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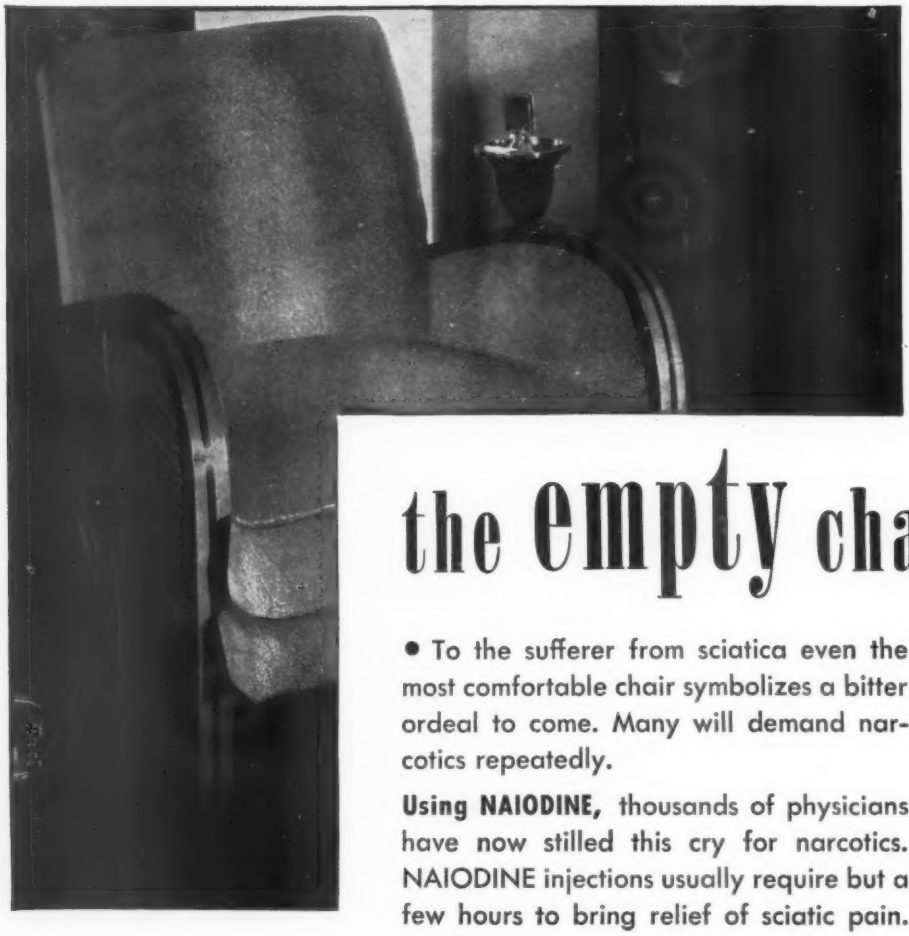

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THE AMERICAN JOURNAL OF THE MEDICAL SCIENCES

AUGUST, 1946

ORIGINAL ARTICLES

THE LEUKOCYTIC RESPONSE OF PATIENTS WITH EXPERIMENTALLY INDUCED INFECTIOUS HEPATITIS*

By Maj. Walter P. Havens, Jr., M.C., A.U.S.
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The leukocytic response of patients with infectious hepatitis has been described as *leukopenia* with a decrease of all the components of the leukocytes, except the monocytes. Some observers[7] have reported an early transient leukocytosis, with leukopenia becoming evident at the time of the appearance of jaundice; others[16,18] have stated that leukopenia is an early phenomenon appearing in the pre-icteric period. A frequently observed feature of this disease has been a *relative lymphocytosis* developing subsequent to an early lymphopenia. Of particular interest in this regard has been the appearance of numerous large mononuclear cells defined variously as immature or atypical lymphocytes, or atypical monocytes.[7,16,18] The association of such cells with the posterior cervical adenopathy, so commonly observed in infectious hepatitis, has been the subject of discussion.[1]

Aside from the broad general association of pyogenic infections with leukocytosis and virus infections with leukopenia, the pattern of leukocytic response in various acute infections is limited. Quantitative alterations in the various components of the leukocytes, particularly the early leukopenia with neutropenia and lymphopenia, similar to those found in infectious hepatitis, have been observed in several acute infectious diseases caused by rickettsia and viruses.[17] Such changes have been described as occurring in measles,[2] pappataci fever,[13] dengue,[12,14] varicella,[6] smallpox,[17] yellow fever,[3] influenza,[17] rubella,[9] typhus fever,[8] and psittacosis.[11]

Qualitative changes with the appearance of atypical lymphocytes similar to those seen in patients with infectious hepatitis have also been reported as occurring in measles[2] and German measles.[2,15] A further comparison of such cells with those ordinarily considered as pathognomonic of infectious mononucleosis[2] has been made.

During the past 18 months, experiments conducted by the Neurotropic Virus Dis-

* Representing work done for the Neurotropic Virus Disease Commission of the Board for the Investigation and Control of Influenza and Other Epidemic Diseases in the Army, in the Preventive Medicine Service of the Office of The Surgeon General, U. S. Army, Washington, D. C.

Acknowledgment is made of the assistance and coöperation of the following agencies: Selective Service, Camp Operations Division; Civilian Public Service Unit No. 68; Civilian Public Service Unit No. 81; Civilian Public Service Unit No. 140; the Connecticut State Hospital, Middletown, Connecticut; the Norwich State Hospital, Norwich, Connecticut; the Federal Correctional Institution, Danbury, Connecticut; and the U. S. Public Health Service.

ease Commission, on the transmission of infectious hepatitis to human volunteers[10] have made it possible to study the hematologic response of such patients throughout the incubation period and course of disease. It is the purpose of this paper to report on the leukocytic changes observed in 26 patients during the course of experimentally induced hepatitis. In 10 of these volunteers similar observations were made at regular intervals throughout the period of incubation as well as during the course of disease.

Materials and Methods. *Subjects.* The subjects were previously healthy, male, human volunteers, ranging in age from 19 to 29 years. These men contracted infectious hepatitis experimentally, following inoculation or ingestion of material known to contain the etiologic agent of the disease. Prior to the onset of disease they were housed in 3 groups: in Middletown, Danbury, and New Haven. During the course of illness all patients except those at Danbury were hospitalized in the Isolation Pavilion of the New Haven Hospital. The group at Danbury was hospitalized at that institution. The diagnosis of infectious hepatitis was made on the basis of characteristic symptoms and signs, accompanied by fever and consistent deflection of the bromsulfalein dye retention and cephalin-cholesterol flocculation tests. All patients mentioned in this report had clinical jaundice.

Virus. The strain of virus used in this laboratory was originally obtained from the stool of a U. S. Army soldier (BE), who contracted *epidemic infectious hepatitis* in Sicily in September, 1943.[5] It has been through 4 passages in human volunteers to date. This agent is filtrable through an L2 Chamberland filter and withstands heating to 56° C. for at least 30 minutes.[4] It has produced the disease in 27 out of 40 human volunteers (including those reported here) following parenteral or oral inoculation with incubation periods ranging from 15 to 34 days.

Blood Counts. 600 total leukocyte and differential counts were made on the 26 human volunteers. Blood counts were made between 9 and 10 A.M., after breakfast. The usual method for determination of the total number of leukocytes was employed. Blood obtained from the finger-tip was diluted in standardized pipettes and shaken for 3 minutes by hand. The number of cells in the 4 corner squares of a standard hemocytometer was counted. Differential counts of fixed smears were made on slides or cover-slips stained with the May-Greenwald-Giemsa technique. 400 to 600 cells were counted and the absolute values were determined. A mechanical stage was used. Care was taken to avoid the periphery of the slides where the smears were very thick and likewise the center of the cover-slips where the greatest pressure has occurred in preparation.

The range and average determinations of the various components of the leukocytes for these 26 human volunteers were computed before the experimental inoculations. Four total counts and differential smears were examined for each man over a period of several days. The results appear in Table 1 and are used as the averages against which the deflections of individual responses during incubation period and course of disease are plotted in the various charts.

TABLE 1.—LEUKOCYTE PICTURE IN 26 HEALTHY HUMAN VOLUNTEERS BEFORE EXPERIMENTAL INOCULATION WITH "VIRUS" OF INFECTIOUS HEPATITIS

Leukocytes	Number / cu.mm. Range	Average
Total	4500-10,900	6700
Neutrophils	1990- 7,400	4100
Seg. neutrophils	1930- 6,400	3650
Non-seg. neutrophils	60- 1,420	450
Lymphocytes	1440- 3,160	2160
Large lymphocytes	120- 980	400
Eosinophils	50- 600	160
Monocytes	90- 490	240
Basophils	0- 180	40

In these experiments leukocyte counts were made every other day throughout the incubation period and almost daily during the first 3 weeks of disease in 10 patients. In the other 16 patients similar studies were made every other day during the first 3 weeks of disease. During the 4th week all 26 patients were examined every 2 to 4 days.

Results. When the leukocytes are counted frequently a regular pattern of response in infectious hepatitis is observed. These characteristic changes usually begin in the first 24 to 48 hours of the acute, febrile, *pre-icteric* phase of disease.

and are primarily associated with the presence of fever. The 26 patients reported here may be almost equally divided into 2 groups. One group had an acute onset of disease with fever. The other group had an insidious onset with vague abdominal symptoms for periods of 2 to 11 days before the appearance of fever. In general, it was observed that the 12 patients who made up the former group manifested the characteristic leukocytic response within the first 24 to 48 hours minor leukocytic changes in the presence of symptoms 2 to 3 days before fever.

By using the appearance of fever as the fixed point, it is possible to present the combined leukocytic response in all 26 patients of this series. When this is done and the average duration of fever and jaundice, and the appearance time of jaundice are recorded it is seen that defervescence, the appearance of jaundice, and a *beginning* approach to normal leukocytic relationship occur at the end of the 1st

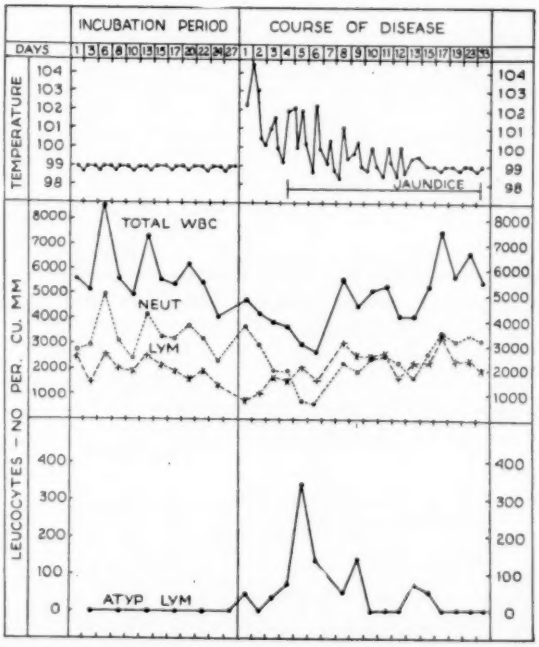

FIG. 1.—Incubation period and course of disease of a patient (SR) with experimentally induced infectious hepatitis. Rectal temperatures are recorded.

of disease, although minor changes might appear in certain individuals in the last 2 to 3 days of the incubation period. (Fig. 1.) In contrast it was evident that the 14 patients in the latter group who had a vague onset with mild generalized or abdominal complaints and a normal temperature usually did not develop the characteristic pattern of change until several days later coincident with the appearance of fever (Fig. 2). However, occasional individuals in this group had week of fever (Fig. 3). In general, it may be said that the early dramatic changes in leukocytic response (leukopenia, lymphopenia and neutropenia) are almost over by the time jaundice appears, although in some patients in whom jaundice appears as early as on the 3rd to 4th day of fever, many of the striking changes are still present (Fig. 1). In certain individual cases, also, a mild relative lymphocytosis with a small percentage of atypical cells continues during the 2nd week, after

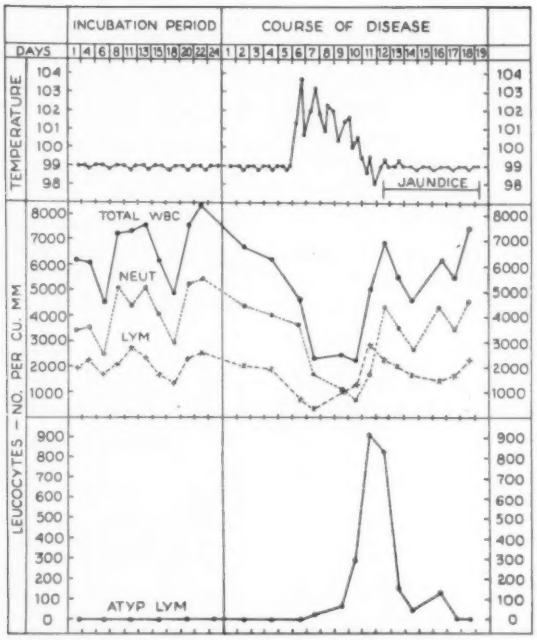

FIG. 2.—Incubation period and course of disease of a patient (SD) with experimentally induced infectious hepatitis. Rectal temperatures are recorded.

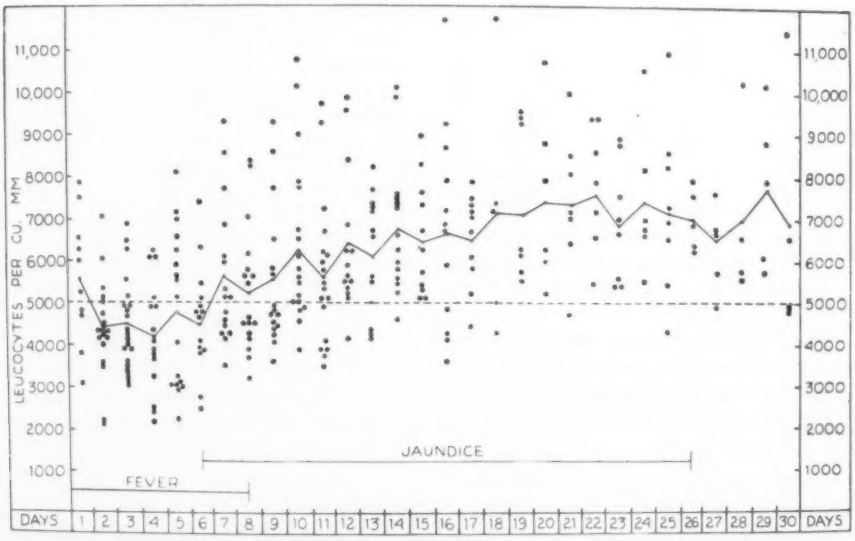

FIG. 3.—Averaged leukocytic response of 26 patients with experimentally induced infectious hepatitis for 30 days after the onset of fever. Black dots indicate individual total leukocyte counts, which are expressed as an average by the unbroken line. The dotted line indicates leukopenic level. The duration of fever and jaundice and the appearance time of jaundice represent averaged experience of the 26 patients.

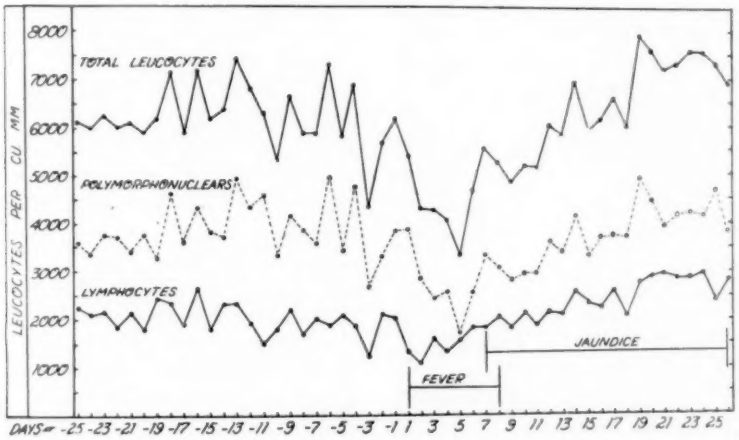

FIG. 4.—Averaged leukocytic response of 10 patients during the incubation period and course of disease of experimentally induced infectious hepatitis. (−) indicates days before onset of fever. The duration of fever and jaundice and the appearance time of jaundice represent averaged experiences of the 10 patients.

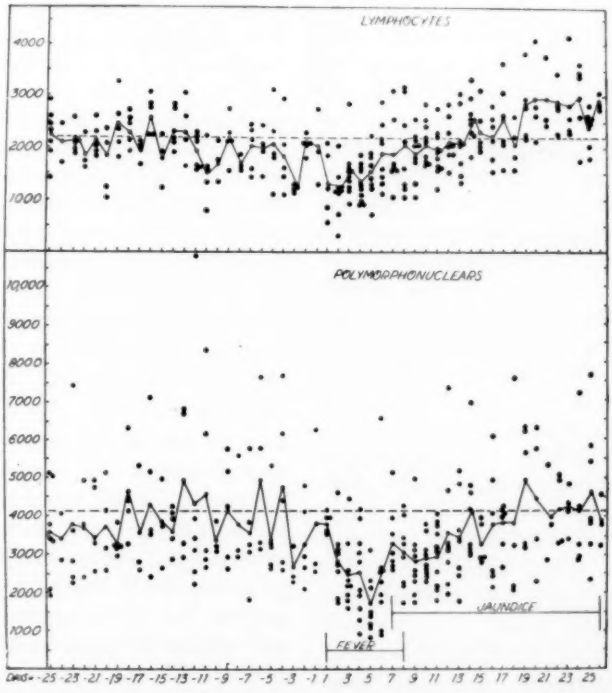

FIG. 5.—Leukocytic response of 10 patients during the course of disease of experimentally induced infectious hepatitis. (−) indicates days before onset of fever. The duration of fever and jaundice and the appearance time of jaundice represent averaged experiences of the 10 patients. Black dots indicate individual determinations which are expressed as an average by the unbroken line. Dotted lines indicate the average normals for the group.

the appearance of jaundice, and in an occasional case a secondary dromedary type of curve similar to the first response occurs in the 2nd or 3rd week (Fig. 1).

For purposes of convenience the leukocytic changes are described as occurring in 3 phases, namely, (1) the incubation (pre-febrile) phase, (2) the febrile (pre-icteric) phase and (3) the icteric (post-febrile) phase.

Incubation (Pre-febrile) Phase. When incubation periods were measured from the day of inoculation to the first appearance of symptoms they ranged from 15 to 28 days. However, the time interval from day of inoculation to *onset of fever* in these patients ranged from 24 to 30 days. Using the onset of fever as a fixed point, the averages of the total number of leukocytes and their individual components were computed for 25 days before fever and 26 days after fever in 10 patients. (Figs. 4 and 5.)

In general there is little fluctuation from normal during the pre-febrile period up until 2 to 3 days before the onset of fever. During the 4th week, before fever, the total number of leukocytes is slightly below the normal average for the group (6700 per c.mm.). Both neutrophils and lymphocytes are diminished in proportion. In the 3rd week, before fever, the total leukocyte count is slightly above normal limits and there is a slight average increase of neutrophils with a slight decrease in lymphocytes. This average increase of total leukocytes is maintained during the 2nd week, along with an increase in neutrophils and decrease of lymphocytes. *Three days* before the appearance of fever the total number of leukocytes drops below the established normal for this group (6700 cells per c.mm.). At this time total counts as low as 4000 cells per c.mm. occasionally occur. This decrease in absolute numbers is shared proportionately by the neutrophils, eosinophils, basophils and lymphocytes. The monocytes are not affected. Such leukopenia before the onset of fever may or may not be accompanied by symptoms, depending on whether it occurs in a patient with an acute or insidious onset of disease.

Febrile (Pre-icteric) Phase. The duration of fever ranged from 4 to 14 days, averaging 8 days. Clinical jaundice ranged in appearance time from 3 to 12 days after the beginning of fever, averaging 6 days (Fig. 3). In all but 1 patient leukopenia, *i. e.*, a reduction to below 5000 leukocytes per c.mm., was found at some time during this febrile pre-icteric phase of disease. The total number of leukocytes ranged between 2250 and 9400 per c.mm. Characteristically leukopenia occurred early, appearing in 25 patients during the 1st 24 to 48 hours of fever, becoming more pronounced until the 4th or 5th day when the low level was reached. The total number of leukocytes began to increase through the 5th and 6th days, rising above the level of leukopenia on the 7th day.

Associated with the leukopenia are certain quantitative changes in the various components of the leukocytes.

Neutrophils. On the 1st day of fever there is a *relative* increase in neutrophils in the presence of leukopenia or low-normal total leukocyte count. Although the absolute number of neutrophils is less than normal, this *relative* increase is possible because of a coincident lymphopenia. By the 2nd day absolute neutropenia is present and this progresses, reaching a low point on the 5th day of fever. Absolute numbers as low as 820 cells per c.mm. has been recorded. On the 6th and 7th days the neutrophils rise sharply although they do not reach the normal level for the group. (Figs. 4 and 5.) During the 1st 5 days of fever, as neutropenia increases, the non-segmented cells increase somewhat in absolute value, but due to the marked decrease in segmented cells, they may number as many as 50% of the total neutrophil count. After the 5th day this relationship is changed and the absolute value of segmented cells rises sharply on the 6th and 7th days, so that the mature cells become again predominant. (Fig. 6.)

Eosinophils. Eosinophilic granulocytes are diminished in absolute numbers during the acute febrile phase of the disease. They are below normal on the 1st day of fever, decline to a low level on the 3rd day, which is maintained until the 5th day after which a slight increase occurs on the 6th to 8th day, although a normal level is not reached.

Basophils. The change in absolute numbers of basophils is not great, but these cells are diminished during the febrile phase.

Monocytes. The absolute numbers of monocytes are sharply depressed during the febrile phase.

The lymphopenia of the 1st 5 days of the febrile period is largely at the expense of the small round cells. The large lymphocytes increase somewhat during this period and, because of the decrease in small cells, may form as many as 40 to 70% of the total number of lymphocytes. Characteristically, on the 1st or 2nd day of fever a few of these large lymphocytes are atypical in appearance. These atypical cells increase in number so that by the 5th day a majority of the large cells may be atypical (Figs. 1 and 2). It is at this time when there may be neutropenia and *relative* lymphocytosis with a predominance of large atypical

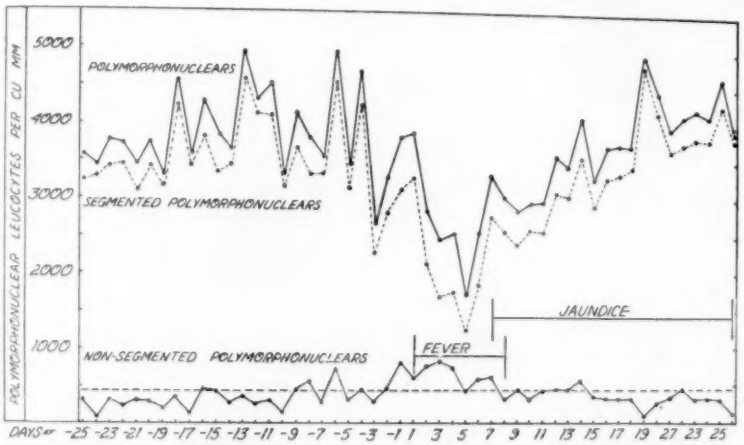

FIG. 6.—Averaged polymorphonuclear leukocytic response of 10 patients during the incubation period and course of disease of experimentally induced infectious hepatitis. (−) indicates days before onset of fever. The duration of fever and jaundice and the appearance time of jaundice represent averaged experiences of the 10 patients. Dotted line indicates average normal level of non-segmented polymorphonuclears for the group.

Lymphocytes. On the 1st day of fever both absolute and relative lymphopenia is present. This reaches a low point on the 2nd day, persisting at a low level through the 4th day. By the 5th day when neutropenia is most marked, the lymphocytes begin to increase, equalling or outnumbering the neutrophils and resulting often in a relative lymphocytosis. An absolute lymphocytosis rarely occurs. The absolute number of lymphocytes continue to rise through the 6th to 8th day although a normal level is not reached.

lymphocytes that considerable difficulty may be encountered in differentiating this leukocytic response from that found in infectious mononucleosis. This is particularly true when the posterior cervical lymphadenopathy, as commonly observed in this phase of infectious hepatitis is present,[1] and jaundice has not yet appeared. From the 5th to the 7th days the number of small lymphocytes increases and they again become predominant.

These atypical cells may be described as large lymphocytes with big eccentri-

cally placed indented nuclei. At times an area of hyaloplasm containing an azure granule is seen at the indentation of the nucleus. The cytoplasm is usually heavily basophilic and the chromatin of the nuclei is arranged in heavily staining bands. Vacuoles are frequently found in both the cytoplasm and nuclei. Other large atypical cells may have a paler blue staining cytoplasm containing numerous coarse, azurophil granules. Such cells frequently have heavy blocks of chromatin in the nuclei. (Fig. 7.)

Icteric (Post-febrile) Phase. The duration of jaundice ranged from 8 to 31 days, with an average of 20 days. Characteristically, clinical jaundice appears with defervescence at the end of the 1st week. At this time the trend of the leukocytic response is in the direction of normal relationships. During the 2nd week the total number of leukocytes rises gradually,

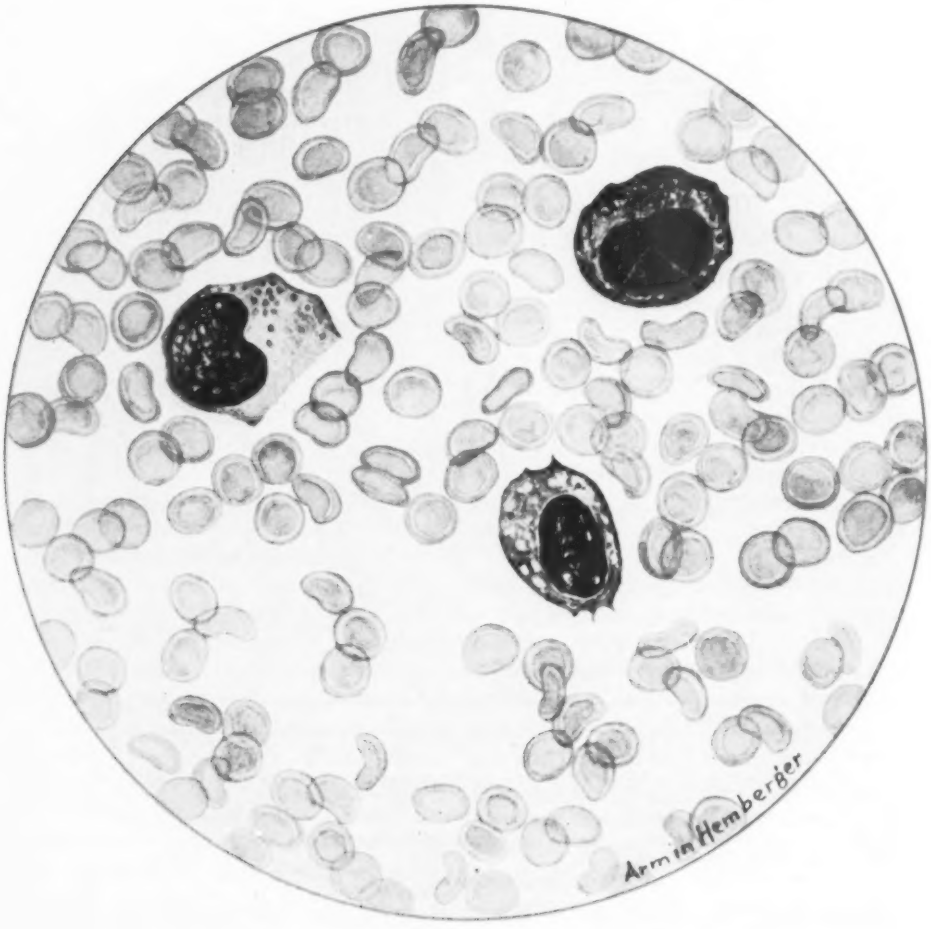

FIG. 7.—Lymphocytes found in the blood on the 5th day of fever of a patient with experimentally induced infectious hepatitis. (Photograph of a colored drawing by Armin Hemberger.) Giemsa-May-Greenwald stain. (× 1125.)

reaching the average normal level of the group on the 14th day (Figs. 3 and 5). This increase usually reflects a proportional increase in all the various components of the leukocytes although important variations may occur. Post-febrile leukocytosis as high as 12,500 cells per c.mm. has been seen. Not infrequently a relative lymphocytosis may persist during the 2nd week with a small proportion of large atypical cells evident, although the small lymphocytes are predominant. Also, on occasion, a secondary "camel" type of curve of lesser degree but resembling the acute changes found in the 1st week may occur (Fig. 1). During the 3rd and 4th week as jaundice reaches its peak, wanes and disappears, the number of leukocytes and the relations of their various components is usually within normal limits.

Discussion. It has been pointed out in this paper that a regular pattern of leukocytic response occurs during the course of infectious hepatitis *experimentally induced* in human volunteers. This is in general agreement with the findings of others working with the naturally occurring disease and quite similar to the response observed in a number of acute infectious diseases caused by viruses and Rickettsia. The latter point may be of some importance as a further bit of evidence for the classification of infectious hepatitis as a disease of "virus" etiology.

It is of interest to note that although slight changes in the leukocytes may occur in individual patients in the last 2 to 3 days before the onset of fever, the characteristic leukopenic response is related primarily to the febrile, pre-icteric phase. Jaundice, which occurs at the end of the 1st week is associated in general with defervescence and a beginning return to normal leukocytic relationships. This is analogous to the situation in measles in which similar changes have been observed associated with the appearance of fever.[2]

The similarity between certain atypical lymphocytes found in patients with infectious hepatitis, measles, German measles, and infectious mononucleosis has been indicated. The fact that such similar cells appear in several diseases of different etiology suggests that their production represents a limited response to a broad group of acute infectious diseases. This is of some practical importance in differential diagnosis, particularly as it concerns infectious hepatitis and infectious mononucleosis. Not infrequently the appearance of a large percentage of atypical lymphocytes in a patient during the febrile, pre-icteric phase of infectious hepatitis causes confusion. This is particularly true when there is an associated enlargement of the posterior cervical glands.

Summary. 1. The results of serial observations on the leukocytic response of patients during the incubation period and course of disease of *experimentally induced* infectious hepatitis are presented.

2. Although minor changes in leukocytes may occur in the last 2 to 3 days before the onset of fever, the characteristic leukocytic response begins in the first 24 to 48 hours of fever and is associated primarily with the febrile, pre-icteric phase of disease.

3. The pattern of this leukocytic response is characterized by the early appearance of leukopenia: lymphopenia and neutropenia. Subsequent relative lymphocytosis with numerous atypical lymphocytes is a common phenomenon.

4. Defervescence, the appearance of clinical jaundice and the trend towards normal leukocytic relationships are commonly associated with the end of the 1st week of fever.

5. The return to normal leukocytic relationships is completed by the end of the 2nd week after the appearance of fever.

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RETROPERITONEAL SPLENOMEGALY

OCCURRENCE IN A CASE OF LEUKOPENIC PLASMA CELL LEUKEMIA

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IN contrast to the enormous literature on the wandering or movable spleen, that on the retroperitoneal spleen is extremely meager. The reasons for this are obvious: the movable spleen is not rare and is easily felt on palpation, although many instances are recorded of its being mistaken for other intra-abdominal masses, even for a pelvic tumor or a pregnant uterus. Also, the movability itself tends to splenic enlargement from congestion and even torsion of the lengthened pedicle may occur. A wholly retroperitoneal position of the spleen is apparently very rare, although the paucity of symptoms and difficulty of recognition may enter into this view.

Developmentally, the spleen originates in the posterior mesogastrium and acquires a complete peritoneal covering. An eventual retroperitoneal position is a distinct developmental abnormality. In the retroperitoneal position the anatomic relations of the organ differ from those found in the normal in that it tends to rest upon the upper pole of the left kidney and posterior to the colon. This relationship to the kidney may explain one of the disorders seen in individuals with the spleen in the retroperitoneal position; that to the colon contributes to the difficulty of identification by physical examination.

The occasional location of the spleen in a retroperitoneal position is mentioned in many books on anatomy and medicine; but a careful search of the literature reveals practically no references to this subject and no report of a case such as is to be detailed here. Spencer[2] has reported a "Case of retroperitoneal prolapse of the spleen into the left loin." The patient, a woman of 32 years, had suffered 9 attacks of pain in the left loin with vomiting, and had the feeling that in that region a "lump" came and went. On examination, there was found deep tenderness and a mass behind the colon. It was noted that normal splenic dullness was not present. The Roentgen ray revealed the kidney and a shadow above it, which was thought to be a hydronephrotic upper pole. Intending to expose the kidney, the surgeon came upon the spleen. This was removed and was found to be 2 or 3 times the normal size. After the splenectomy it was seen that the kidney had been pushed downward by the overlying spleen. No explanation of the symptoms nor the enlargement of the spleen was ventured, no postoperative history given.

Two somewhat similar patients are reported by Ehrich,[1] in each of whom there was left hydronephrosis. At operation, the spleen was found behind the peritoneum in a dense common capsule with the kidney. These cases seem to suggest that hydronephrosis may be associated with a retroperitoneal position of the spleen, perhaps as a result of displacement of the kidney downward with kinking of the ureter.

In the unique case now to be recorded, the diagnosis of an unusual type of leukopenic (aleukemic) leukemia was made more difficult by the presence of a large mass in the left renal region which defied identification as spleen—a fact which was explained at the autopsy when an enlarged retroperitoneal spleen was found.

Case Report (Hosp. U. of Pa. 44–68456). The patient, a male of 63 years, commenced

to feel fatigued and weak 4 years before admission on Oct. 3, 1944. Six months before this date, he received, in another hospital, 20 blood transfusions for anemia and further transfusions recently in the same institution. He was carefully studied there and the bone marrow was found to be infiltrated with large (possibly malignant) unidentifiable cells. No primary neoplasm could be found and the final diagnosis was aplastic anemia (etiology unknown). In the month before admission, he lost weight rapidly and had irregular fever. Except for diabetes mellitus his past medical history was negative.

The patient's physical condition was very poor with evident pallor and emaciation. A slight but widespread enlargement of palpable lymph nodes was present. On inspiration the liver edge was felt 5 cm. below the rib margin. In the left kidney region, the kidney could be identified, and, with difficulty, another mass could be felt lying more posteriorly than anteriorly and not obliterating the tympany of Traube's semilunar space. This mass presented no edge and only came down about 2 fingers' breadth below the costal margin on deep inspiration. On the skin of the thigh were several indurated, palm-sized plaques which were diagnosed as areas of leukemic infiltration by the dermatologic consultant.

Of the extensive laboratory studies, only the following need be detailed: in the peripheral blood, a severe normocytic anemia with the erythrocyte count varying a little above or below 2,000,000 and the leukocytes ranging between 1000 and 3000. There was a reduction in neutrophils and a resulting high percentage of lymphocytes. No abnormal or immature forms of white blood cells were seen until terminally. Three plasma cells were noted on 1 occasion. The thrombocyte count varied from a normal to a low level. A careful study of the bone marrow obtained by sternal puncture led, for the second time, to the opinion that the numerous large, vesicular, nucleated cells were evidence of malignant infiltration and that the process was not leukemia. The usual evidence of mild diabetes mellitus was present.

Pursuing the above suggestion, a second search for a primary neoplasm was carried out. Roentgenograms of chest, gastro-intestinal tract, urinary tract, and most of the skeleton revealed nothing abnormal except confirmatory evidence that there was a large abnormal mass in the left kidney region pushing the diaphragm up and the kidney down (Fig. 1). In consultation with the roentgenologists, they pointed out that if this mass were spleen it should be more easily palpable; that it lay above the kidney pushing the diaphragm upward. The suggestion was made that it might be a retroperitoneal neoplasm such as hypernephroma.

In addition to his progressive anemia and wasting, the patient had a mild diabetes which was controlled with 10 units of insulin daily. He also had many subcutaneous abscesses requiring drainage. These infections and his poor general condition made biopsy of a lymph node unwise. In spite of transfusions, penicillin and general care, the patient failed rapidly and died on Nov. 25, 1944, after 7 weeks stay in the hospital.

The diagnostic problem presented by the patient was an interesting one. It was believed that the presence of diabetes was a coincidence and could be disregarded. Essentially the diagnosis rested on the identification of the abnormal cells in the bone marrow and of the abnormal mass above the left kidney. The anemia could be explained by the heavy infiltration of the marrow with abnormal cells, whether of neoplastic or leukemic nature. Several pathologists and hematologists agreed that these cells were malignant and not leukemic. If this opinion were correct, it must argue strongly for the neoplastic nature of the mass above the left kidney. If, however, the abnormal cells were leukemic, then an enlarged spleen might well be anticipated. The mass, however, did not offer the usual criteria for recognition of an enlarged spleen. No edge or notch could be felt; it was not in the usual position, it did not obliterate Traube's area of tympany, the colon lay in front of it and the kidney was pushed down by it. These were our views at the time of the patient's death; no one had suspected a retroperitoneal splenomegaly.

Autopsy. (J. Snyder—7 hours after death.) As is so often the case in the elderly, there were present many pathologic findings which apparently bore no immediate relation to the disease causing death. In this 63 year old asthenic individual, there were found a dissecting aneurysm of the aorta, thrombosis in the right auricle and vena cava, nephrosclerosis and necrosis of the esophageal mucosa. The pertinent findings in-

cluded widespread leukemic infiltration of bone marrow, lymph nodes, liver and spleen with cells of the plasma cell variety. This clinched the primary diagnosis and leaves for discussion the abnormal mass in the left kidney region. This mass proved to be abnormality of the kidney. Under the microscope, it was seen that "the norma architecture of the spleen has been defi nitely disrupted and the spleen tissue con sists merely of a diffuse cellular tissue show_ ing a majority of plasma cells with lympho_

FIG. 1.—Film from urogram series showing enlarged retroperitoneal spleen pressing down on left kidney.

a large completely retroperitoneal spleen, weighing 650 gm., enclosed in a well-fitted peritoneal capsule. It was somewhat oval in shape, bluish red in color and increased in consistency. Although the left kidney was pushed down by the spleen, there was no evidence of hydronephrosis or any gross cytes seen in the minority." This same picture was seen in the lymph nodes and bone marrow and to a lesser degree in the liver.

Discussion. From this brief review of the literature and this case report, it would

seem that the following statements can be made: (1) The spleen, as a result of a rare developmental anomaly may occupy a wholly retroperitoneal position. (2) The retroperitoneal spleen may cause no symptoms but occasionally may be associated with left hydronephrosis and may perhaps cause this condition by displacement of the kidney downward. (3) A retroperitoneal spleen may be enlarged in any of the diseases which cause splenomegaly; when this occurs the resulting mass is difficult to identify by physical examination or roentgenography. (4) The enlarged retroperitoneal spleen pushes the left diaphragm upward and the left kidney downward; it lies posterior to the stomach and the colon and does not obliterate the tympany of Traube's semilunar space; it fails to present to palpation the characteristic splenic edge or notches.

REFERENCES

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A MODIFIED CEPHALIN CHOLESTEROL TEST (HANGER) IN THE STUDY OF HEPATIC DISEASE*

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As devised by Hanger,[4,5] the cephalin cholesterol flocculation test has proved to be a valuable tool in the study of liver disease. There are, however, certain inherent difficulties in the procedure which have led to irregular results.[2,3,9,12,13,14,16] Thus, if ripe cephalin was utilized, false negative tests were obtained, whereas "ripening" of the cephalin by exposure to sunlight for a few weeks caused false positive reactions.[9,15] Further investigation[9] revealed that prolonged exposure of the antigen to sunlight eliminated some flocculation with normal sera. Apparently the ripening process tends at first to increase markedly the cephalin sensitivity, and later to decrease it for some reason or other.[8] The effect of variability in sensitivity of the emulsion on the final result is illustrated by the recent report of Lippincott *et al.*,[7] who found that tests with serum from acute cases of relapsing vivax malaria yielded 82% positive reactions with one cephalin cholesterol emulsion, whereas tests with another antigen were positive with only 21% of the same sera. The Hanger procedure is further complicated by the fact that fresh sera must be used,[5] preferably samples drawn within 6 hours of testing.[2,14,16] More recently the studies of Neefe and Reinhold,[12] and also Mateer[8] have emphasized photosensitivity as a source of some false positive reactions. Thus normal serum antigen mixtures exposed to sunlight or to an incandescent lamp for a period of 48 hours almost invariably flocculated while duplicate specimens kept in the dark remained in homogeneous suspension. They[12] further found that if normal serum was permitted to stand diluted for 5 or more hours prior to the addition of emulsion, flocculation occurred. Conducting the test at 37° C. resulted, in some instances, in flocculation although no reaction was noted at 21° C.[2,12]

The objective of the present investigation has been to obtain a stable but sensitive sterol emulsion which could be utilized for the examination of serum in a manner similar to that used in serologic tests.

Methods. *Preparation of Hanger Antigen (H).* Thirty-five ml. of redistilled water were heated to 70° C. in a 150 ml. Erlenmeyer flash. One ml. of stock etherized cephalin cholesterol mixture (Difco)† was added dropwise and with agitation. The emulsion was then permitted to boil slowly until the total volume had been reduced to 30 ml., gradually cooled to room temperature, and utilized in the test.[5]‡

Preparation of the Modified Antigen (M). One ml. of stock etherized cephalin cholesterol mixture (Difco) was added dropwise and with agitation to 35 ml. of redistilled water at *room temperature* (20° to 25° C.). The emulsion was then permitted to boil slowly until the total volume had been reduced to 30 ml., gradually cooled to room temperature, and utilized in the test.

The 2 antigens differed considerably in

* These investigations were aided through the Commission on Influenza, Board for the Investigation and Control of Influenza and Other Epidemic Diseases in the Army, Preventive Medicine Service, Office of The Surgeon General, U. S. A.

† Reduction in volume of the emulsions was accomplished by placing the flasks on a hot-plate and boiling gently for 6 minutes at 550° F.

‡ Furnished through the courtesy of the Difco Laboratories, Inc.

appearance and in reaction. The new preparation is more opalescent and the particles are more coarsely dispersed; furthermore, serum sterol mixtures produced a granular precipitate rather than the flocculent type usually obtained with the Hanger antigen.

Performance of the Standard Test. Two-tenths ml. of serum was added to 4 ml. of 0.85% saline. Shortly thereafter, 1 ml. of cephalin cholesterol antigen was added, the reagents were thoroughly mixed, placed in the dark room at 20° to 25° C., and examined in subdued light at the end of 24 and 48 hours. Readings from 0 to 4+ were recorded after the method of Hanger.[5]

Performance of Titration. Serial dilutions of serum in 0.85% saline were made in chemically clean serologic tubes as follows: 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256. A single pipette was used for the entire series of dilutions and the final volume in each tube was 0.8 ml.; 0.2 ml. of the modified antigen was then added to all tubes. The mixtures were shaken thoroughly and then placed in the ice-box at 5° C. unless otherwise stated. They were examined 18 to 20 hours later. The intensity of the reaction was indicated as follows: 1+, a readily visible granularity of the emulsion; 2+, an increased granularity with or without slight sedimentation; 3+, heavy agglutination of the particles accompanied by 50 to 75% sedimentation; 4+, complete agglutination and sedimentation of the emulsion. Occasionally, with normal sera the tubes containing the 1:4 and 1:8 dilutions showed simple sedimentation without the characteristic conglomeration of particles when the settled material was stirred up. Such sediments were classed as negative reactions. All titration experiments were carried out with the *M* antigen unless otherwise stated.

Interpretation of Titration Test. The degree of flocculation in each tube of the serial dilution was recorded after incubation at 5° C. for a period of 18 to 20 hours. The total number of plusses was then added and constituted a cumulative score. The maximum score for a 7 tube titration was 28, provided all tubes showed 4+ flocculation. Conversely, the minimum score was 0. For best interpretation of results the degree of flocculation in each tube should be reported together with the cumulative score. For this purpose a graph can be readily constructed.

Results. *Influence of Ageing, Light and Dilution Upon Reactions With Normal Sera.* Three sets of comparative data with sera from 24 individuals who were considered to be entirely well are presented in Table 1. In Experiment A the standard flocculation test was performed with the Hanger antigen and again with the modified cephalin cholesterol emulsion under favorable conditions, namely, fresh, undiluted sera which had been protected from light at all times. One + reactions with either antigen were considered to be within normal limits. Sera 5 and 7 showed 2+ flocculation with the Hanger antigen after 48 hours incubation at room temperature. No significant flocculation occurred with the new antigen under the same conditions.

In Experiment B the sera were diluted and permitted to stand in the sunlight for 6 hours prior to the addition of the lipid emulsions. Using Hanger antigen, within 24 hours 2+ or greater flocculation was evident in 8 specimens and by 48 hours the number had increased to 12. In contrast the modified antigen remained in homogeneous suspension with all 24 sera.

In Experiment C the same sera were retested after storage at 4° to 5° C. for a period of 6 months. By this time 21 specimens gave positive flocculation with Hanger antigen in 24 hours and all but 1 reacted within 48 hours. The altered antigen, however, showed significant flocculation only with Serum 16 at 24 hours and also with Sera 20 and 21 after 48 hours incubation.

Simultaneously the same sera were tested by the serial dilution method in Experiment D. The highest score recorded was 7+ in 2 instances, whereas the serum from a case of acute hepatitis showed a score of 23. These data confirmed the previous observation that the cephalin cholesterol flocculation test performed according to the method of Hanger was not reliable with diluted and light exposed, or with old sera.[8,12,16] They showed, on the other hand, that with the modified antigen these false positive reactions with normal sera were essentially eliminat-

ed, particularly if the titration technique was utilized.

Influence of Temperature of Incubation on Titration Tests With Normal and Abnormal Sera. The effect of varying the temperature of incubation on the serial dilution test is presented in Table 2. Incubation at 5° C. and at 21° C. resulted in strong flocculation with sera from 2 cases of acute hepatitis and weak or no reactions with normal samples. When the temperature was elevated to 37° and 56° C., the differences between hepatitic and normal sera were greatly decreased.

Table 1.—Effect of Age, Light and Dilution on Comparative Cephalin-Cholesterol Flocculation Tests With Normal Sera

Experiment:		A				B				C				D
Antigen:		Hanger		Modified		Hanger		Modified		Hanger		Modified		Modified
Incubation hours:		24	48	24	48	24	48	24	48	24	48	24	48	20
Serum	1	0	0	0	0	3	4	0	0	2	4	0	1	0
	2	0	0	0	0	3	4	0	0	3	4	0	0	0
	3	0	0	0	1	0	1	0	1	4	4	0	0	4
	4	0	0	0	0	0	0	0	0	2	3	0	0	4
	5	0	2	0	0	0	0	0	0	3	4	0	0	1
	6	0	1	0	0	3	4	0	0	4	4	0	0	0
	7	0	2	0	0	1	3	0	0	4	4	0	1	2
	8	0	0	0	1	2	3	0	0	0	0	0	1	4
	9	0	0	0	0	0	0	0	0	4	4	0	0	1
	10	0	0	0	0	1	2	0	0	4	4	0	0	0
	11	0	0	0	0	2	3	0	0	2	4	0	0	1
	12	0	0	0	0	0	0	0	0	3	4	0	0	0
	13	0	0	0	0	0	0	0	0	3	4	0	0	5
	14	0	0	0	0	2	3	0	0	4	4	0	0	4
	15	0	0	0	0	0	0	0	0	1	2	0	0	1
	16	0	0	0	0	0	0	0	0	4	4	4	4	1
	17	0	0	0	0	0	0	0	0	4	4	0	0	5
	18	0	0	0	0	1	2	0	0	1	3	0	1	4
	19	0	0	0	0	0	0	0	0	2	4	0	0	0
	20	0	0	0	1	0	0	0	0	3	4	1	2	5
	21	0	1	1	1	2	3	0	0	4	4	1	2	7
	22	0	0	0	0	1	1	0	0	4	4	0	0	3
	23	1	1	0	0	2	2	0	0	4	4	0	0	7
	24	0	0	0	0	1	2	0	0	4	4	0	0	3
Acute hepatitis:		4	4	3	4	..	..	..	..	4	4	4	4	23

A = Fresh sera, light protected and undiluted. C = Retested after standing at 4° to 5° C. for 6 months.
B = Diluted and exposed to sunlight for 6 hours. D = Tested by the titration method.
The numbers indicate the degree of flocculation except in Experiment D where they represent the cumulative score after 20 hours incubation at 5° C.

A number of freshly drawn normal and abnormal sera were then examined utilizing 5° and 21° C. as incubation temperatures. These data are shown in Table 3. It was noted that there were 2 zones of reaction, each of which was accentuated by the particular temperature of incubation. Both with normal and abnormal specimens 5° C. favored flocculation with serum of lower dilutions, while 21° C. resulted in an increased flocculation in the higher dilutions of serum as illustrated by Specimens 6, 8, 9, 10, 11, 12, 13, 14, and 15. Acute hepatitic sera were usually characterized by the fact that they caused flocculation throughout the entire range of titration at both incubation temperatures. This type of reaction was not entirely limited to hepatitis but also occurred with some sera from other forms of liver disease (Serum 5). The most common pattern seen in convalescent hepatitis, cirrhosis and tumors involving the liver parenchyma was exemplified by Serum 4, namely strong flocculation with serum of low dilution, a narrow zone in which the mixtures remained in homogeneous suspension or were only partially flocculated, followed by an end-zone where stronger reactions again appeared. Normal sera differed in that they showed weak or no reactions when diluted 1 to 10, a broad mid-zone in which flocculation was absent, and an end-zone characterized by slight flocculation after 48 hours at 5° C. which was accentuated by incubation at 21° C.

Standard tests with the Hanger antigen as shown in Table 3 indicated that 8 of 10 patients with hepatic disturbance had

TABLE 2.—EFFECT OF VARYING TEMPERATURE OF INCUBATION ON THE TITRATION TEST

Incubation (° C.)	Sera	Serum dilutions 1						
		4	8	16	32	64	128	256
5	H	4	4	4	3	3	2	1
	H	3	4	4	4	4	4	3
	N	0	0	0	0	0	0	0
	N	0	0	0	0	0	0	0
21	H	4	3	2	2	2	1	1
	H	3	4	4	3	3	3	3
	N	0	0	0	0	0	1	1
	N	0	0	0	0	0	0	0
37	H	1	1	1	1	1	2	2
	H	0	0	1	3	4	4	4
	N	0	0	0	1	4	4	4
	N	0	0	0	0	0	0	2
56	H	0	0	0	0	2	3	4
	H	2	0	0	3	4	4	4
	N	2	0	0	1	3	4	4
	N	2	0	0	2	3	4	4

H = Acute hepatitis. N = Normal. Readings after 20 hours incubation.

TABLE 3.—EFFECT OF INCUBATION AT 5° C. AND 21° C. ON TITRATION TEST WITH NORMAL AND ABNORMAL SERA AS COMPARED WITH STANDARD PROCEDURE

Serum No.	Source	Incubation at 5° C. mod. antigen — Serum dilutions 1									Incubation at 21° C. mod. antigen — Serum dilutions 1									Standard test — Antigen	
		10	20	40	80	160	320	640	1280	2560	10	20	40	80	160	320	640	1280	2560	Hanger	Mod.
1	AH	4*	4	4	4	4	4	4	3	1	4	4	4	4	4	4	4	4	2	4	4
		4†	4	4	4	4	4	4	4	2	4	4	4	4	4	4	4	4	2	4	4
2	AH	4	3	3	4	4	4	3	3	2	4	2	2	2	4	4	4	4	4	4	3
		4	4	4	4	4	4	4	3	2	4	3	3	4	4	4	4	4	4	4	4
3	CH	4	3	2	3	3	2	2	2	1	2	2	2	1	2	2	2	2	2	4	1
		4	4	2	4	4	4	3	3	2	3	2	2	3	4	4	4	4	4	4	3
4	CH	4	3	1	0	0	0	0	1	0	4	3	1	0	0	0	1	2	3	4	0
		4	4	1	0	0	2	2	2	3	4	3	1	0	0	1	2	3	3	4	1
5	AC	4	3	3	4	4	4	3	2	1	4	1	0	1	2	3	3	2	2	4	0
		4	3	3	4	4	4	4	3	2	4	1	1	2	4	3	4	4	4	4	2
6	AC	2	1	0	1	1	1	1	1	1	0	0	0	0	1	3	2	2	2	3	0
		3	1	0	1	2	2	2	2	3	0	0	0	0	2	3	4	3	3	4	0
7	CK	4	4	2	2	0	1	1	1	1	4	3	1	1	2	2	3	3	2	4	4
		4	4	2	2	2	2	2	1	1	4	4	1	1	2	2	3	3	3	4	4
8	CB	4	2	0	1	2	3	2	2	1	1	0	0	0	0	1	2	3	2	4	0
		4	3	0	1	3	3	3	2	2	1	0	0	0	0	2	3	4	3	4	1
9	C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	1	0
		0	0	0	0	0	0	1	1	2	0	0	0	0	0	0	0	2	3	1	0
10	PA	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
		3	0	0	1	1	1	2	2	1	0	0	0	0	0	1	1	1	1	0	0
11	N	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
		1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	2	2	0	1
12	N	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	2	2	1	0
		0	0	0	0	0	0	1	2	1	0	0	0	0	1	1	3	3	3	1	1
13	N	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	2	0	0
		2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	4	3	0	0
14	N	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	2	2	1	0
		2	0	0	0	1	1	1	2	1	0	0	0	0	0	0	2	2	3	1	1
15	N	2	0	0	0	0	0	0	1	0	0	0	0	0	0	0	2	3	2	0	0
		2	0	0	0	0	1	1	1	0	0	0	0	1	2	2	4	4	3	1	1

AH = Acute hepatitis. C = Cholelithiasis. CK = Carcinoma of kidney.
CB = Carcinoma of breast. AC = Alcoholic cirrhosis. N = Normal.
CH = Convalescent hepatitis. PA = Pernicious anemia.
Numbers from 0 to 4 indicate degree of flocculation.
* 24 hour reading.
† 48 hour reading.

parenchymatous involvement. The modified antigen seemed much more discriminating when tested by the same method. Flocculation within 24 hours was limited to sera from the 2 patients with acute hepatitis and to 1 with hepatic metastases. After 48 hours 2 additional positive reactions appeared, 1 with serum from a case of convalescent hepatitis and the other from a patient with alcoholic cirrhosis of the liver. These results are presented primarily to illustrate the effect of temperature over a wide range of dilutions which differ from those employed in the titration test as adopted for routine use.

5° C., flocculation in the fore-zone again became evident although it remained somewhat reduced in the sera heated for 30 or 60 minutes.

Influence of Different Lots of Antigen. The effect of varying the commercial lot of cephalin cholesterol on the titration test is given in Table 5. Within the limits of sensitivity of any given antigen the ability to flocculate appeared to be dependent upon the characteristics of each individual serum. Thus, Emulsion E which showed the strongest reactions throughout, gave relatively weak flocculation with Serum 3 and Serum 7 in which the least reactions

TABLE 4.—EFFECT OF INACTIVATION OF SERUM AT 56° C. ON TITRATION TEST

Inactivation 56° C.	Serum	Serum dilutions 1						
		4	8	16	32	64	128	256
None	AH	3*	4	4	4		4	4
		4†	4	4	4	4	4	4
	CAV	2	2	0	0	0	0	0
		4	4	1	0	0	0	1
	N	0	0	0	0	0	0	0
		3	0	0	0	0	0	0
15 min.	AH	1	1	2	2	4	4	4
		4	4	4	4	4	4	4
	CAV	0	0	0	0	0	0	0
		1	0	0	0	0	0	1
	N	0	0	0	0	0	0	0
		1	0	0	0	0	0	0
30 min.	AH	0	0	1	2	3	3	3
		1	3	4	4	4	4	4
	CAV	0	0	0	0	0	0	0
		0	0	0	0	0	0	0
	N	0	0	0	0	0	0	0
		0	0	0	0	0	0	0
60 min.	AH	0	0	0	0	2	3	3
		1	1	3	4	4	4	4
	CAV	0	0	0	0	0	0	0
		0	0	0	0	0	0	0
	N	0	0	0	0	0	0	0
		0	0	0	0	0	0	0

AH = Acute hepatitis. N = Normal. CAV = Carcinoma ampulla of vater.
* Readings after 20 hours incubation at 5° C.
† Readings after 40 hours incubation at 5° C.

Influence of Preliminary Inactivation of Serum at 56° C. on the Titration Test. The effect of preliminary inactivation of serum at 56° C. upon the serial dilution test incubated at 5° C. is shown in Table 4. Heating for as little as 15 minutes reduced the intensity of the flocculation in the fore-zone of tests read after 20 hours incubation and heating for 60 minutes abolished it completely in both normal and pathologic sera. When the serum-lipid mixtures were permitted to stand for 40 hours at

were obtained with all antigens. The lipid emulsions, A, B and E, were most sensitive whereas C and D yielded weaker flocculation with the same sera. These data indicate that standardization of stock etherized cephalin cholesterol preparations is of prime importance in order to obtain comparable results. Such a procedure is now possible with the modified antigen emulsion which permits utilization of serum for long periods of time. Stored normal and abnormal sera with known

titration patterns may be employed to standardize new lots of antigen. In this manner the wide variations noted in Table 5 with Sera 2, 4, 5 and 6 may be avoided. Those antigens with unusually high or low sensitivity should be discarded.

Observations in the Course of Infectious Hepatitis. During the period of this investigation specimens of serum were obtained from a number of human subjects who were being observed for the development of infectious hepatitis. The flocculation data are presented under 3 headings in Table 6: (1) Standard Tests With the Hanger Antigen "H;" the readings are recorded for 24 hour* periods but 48 hour readings, not included in the table, showed no significant changes. (2) Standard Tests With the Modified Antigen "M"—which were read after 48 hours. The difference between 24 and 48 hour readings were, in a few instances, significant here. Both tests were performed with freshly drawn sera. (3) Titrations Tests "T"—with modified antigen performed when the preinoculation specimens were approximately 7 months old. The results with the standard tests are expressed as 0 to 4+ reactions, whereas the titration data are tabulated as the cumulative score obtained after incubation of the mixtures for 20 hours at 5° C. The appearance of bilirubinemia is indicated by the italic figures of Table 6 utilizing 1 mg. per 100 ml. as the upper limit of normal. Four of the subjects developed clinical jaundice; the remaining patients showed suggestive findings such as anorexia, nausea, vomiting, right upper quadrant tenderness or liver enlargement but laboratory evidence of hepatic disease was not demonstrated. A study of the table reveals that the cephalin

TABLE 5.—TITRATION TESTS WITH FIVE LOTS OF CEPHALIN-CHOLESTEROL ANTIGEN

Serum	Antigen lot	Serum dilutions 1							
		4	8	16	32	64	128	256	Score
1. Acute hepatitis	A	4	4	4	4	4	2	2	24
	B	4	4	4	4	4	3	2	25
	C	4	4	3	3	1	1	0	16
	D	4	4	4	4	3	1	1	21
	E	4	4	4	4	4	4	3	27
2. Cholelithiasis with hepatitis	A	4	0	0	3	2	2	1	12
	B	4	0	0	3	3	2	2	14
	C	3	0	0	0	0	0	0	3
	D	4	0	0	0	1	1	1	7
	E	4	0	0	3	3	3	3	16
3. Carcinoma, ampulla of Vater	A	1	0	0	0	0	0	1	2
	B	2	1	0	0	0	0	0	3
	C	1	0	0	0	0	0	0	1
	D	2	0	0	0	0	0	0	2
	E	1	0	0	0	0	0	1	2
4. Cirrhosis, Banti type	A	4	1	0	0	1	1	1	8
	B	4	2	0	1	3	3	2	15
	C	3	1	0	0	0	0	0	4
	D	4	1	0	0	1	1	0	7
	E	4	1	1	3	4	4	4	21
5. Cirrhosis, portal type	A	2	0	0	1	1	1	1	6
	B	3	1	0	1	2	1	1	9
	C	1	0	0	0	0	0	0	1
	D	2	1	0	0	1	0	0	4
	E	3	0	1	3	3	2	2	14
6. Normal	A	0	0	0	1	1	0	0	2
	B	0	0	0	0	2	2	0	4
	C	0	0	0	0	0	0	0	0
	D	0	0	0	0	1	0	0	1
	E	1	0	1	3	3	1	1	10
7. Normal	A	0	0	0	0	0	0	0	0
	B	1	0	0	0	0	0	0	1
	C	0	0	0	0	0	0	0	0
	D	0	0	0	0	0	0	0	0
	E	0	0	0	0	0	0	0	0

Readings after 18 hours incubation at 5° C.

* The 24 hour reading is preferred by some investigators.

cholesterol flocculation test with the Hangar antigen yielded distinctly positive results with the onset of jaundice. Similarly the new antigen as utilized in the standard test proved to be of approximately the same order of sensitivity except in Case 4 where it caused flocculation during convalescence which was not manifest by the Hanger preparation. The titration tests seemed to follow the course of the hepatitis with greater accuracy and showed progressive changes in the degree of flocculation during the transition from acute illness to convalescence. Furthermore, on the 42nd day following inoculation the titration procedure was positive in Cases 3 and 4 prior to the onset of clinical jaundice while the standard tests showed equivocal results in the form of 2+ reactions which also appeared at times with the sera from patients who did not develop hepatitis (Cases 6 and 8). Although more frequent samplings in the pre-icteric phase would have been advantageous in establishing this point, the data suggested that the titration procedure was at least as sensitive as the standard tests and more readily interpreted. All 3 procedures yielded negative or doubtful results during convalescence even in the presence of clinical jaundice, a finding noted previously by Hangar.[5]

Results of Titration Tests. Summary data showing the cumulative score of normal and abnormal sera are presented in Table 7. For purposes of simplification the sera have been grouped into those with a score of 5 or less, 6 to 10, 11 to 15, and over 15. Among 100 ambulatory adult persons in apparently normal health 95 scored 5 or less in the titration test. The remaining 5% ranged between 6 and 10. A second group of 100 sera were obtained from patients in hospital who were suffering from illnesses other than liver disease. Of these individuals, 98% scored 10 or less in the flocculation titration test. From the 2 persons with scores exceeding 15, second specimens of serum were obtained and both scored 8 on retesting. The term "infected but not ill" refers to 11 subjects who received potentially ictero-

TABLE 6.—STANDARD AND TITRATION FLOCCULATION TESTS IN INFECTIOUS HEPATITIS

Case	Antigen	Days from inoculation: 0	10	21	31	42	46	52	56	63	73	84	94	105	115
1	H	0	0	0	0	4									
	M	0	0	0	0	3									
	T	0	0	0	0	*24*									
2	H	0	0	0	0	3	3	4	4	0	0	0	0	0	0
	M	1	0	0	0	2	2	3	0	0	0	0	0	2	0
	T	2	0	1	6	*25*	*21*	*23*	*9*	*0*	*0*	0	1	0	5
3	H	0	0	0	0	2	3	3	4	0	0	0	0	0	0
	M	0	0	0	0	0	3	4	3	1	0	0	0	0	0
	T	0	0	0	2	21	*28*	*20*	*10*	*1*	*0*	*0*	0	0	0
4	H	0	2	1	0	2	4	4	4	4	0	0	0	0	0
	M	0	0	1	0	2	2	3	4	3	1	3	0	3	0
	T	1	3	5	7	13	*18*	*28*	*27*	*19*	*9*	*8*	*7*	*10*	3
5	H	0	0	0	0	0	0	0	3	0	0	0	0	0	0
	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	T	2	0	0	1	3	2	0	1	4	2	2	4	6	0
6	H	0	0	0	0	0	0	0	2	0	0	0	0	0	0
	M	0	0	0	0	0	0	2	0	0	0	0	0	0	0
	T	2	0	1	1	2	0	0	2	0	1	1	3	7	
7	H	0	0	0	0	0	0	0	1	0	0	0	0	0	0
	M	0	0	0	0	1	0	0	0	0	0	0	0	0	0
	T	3	..	4	4	6	2	4	1	1	3	5	4	5	8
8	H	0	1	0	0	2	2	0	1	0	1	0	0	2	3
	M	1	0	0	0	0	1	2	0	0	1	0	2	1	0
	T	1	1	3	2	1	3	1	0	2	2	6	1	6	2

H = Hanger antigen; 24 hour reading expressed 0 to 4+.
M = Modified antigen; 48 hour reading expressed 0 to 4+.
T = Titration; 20 hour reading at 5° C. expressed as cumulative score from 7 tube test with an initial serum dilution of 1 to 4.
The italic figures represents the period during which the blood bilirubin exceeded 1 mg. per 100 ml.

genic material but did not develop hepatitis. Serum was obtained at approximately 10 day intervals for a period of 5 months and finally examined by the serial dilution method. All 98 specimens were scored as 10 or less with the procedure used. The above 3 groups of individuals were utilized for the purpose of establishing the range and degree of flocculation which could be expected in normal persons and in patients with diseases other than those involving the liver. From these data it was concluded that a score of 0 to 5 with a lipid emulsion of known sensitivity was normal; scores of 6 to 10 were classed as doubtful and worthy of further study.

a particular serum to flocculate the lipid emulsion. Grouping of convalescent hepatitic sera in relation to the day of illness revealed that after 15 to 30 days, flocculation scores exceeding 11 were obtained in 33%. Sera drawn from 31 to 60 days after the onset yielded positive reactions in 22%; and samples 60 days after illness scored less than 5 in all instances. These findings are in agreement with those obtained with the usual Hanger procedure in that flocculation tended to decrease as convalescence progressed.

Discussion. Attempts to utilize a dilution method in the cephalin cholesterol flocculation test have been previously reported.[1,10] In 1943 Bruger[1] suggested that

TABLE 7.—SUMMARY DATA OF TITRATION TESTS WITH NORMAL AND ABNORMAL SERA

Source of serum samples	No. cases	No. specimens	Cumulative score: 0 to 5 No.	0 to 5 %	6 to 10 No.	6 to 10 %	11 to 15 No.	11 to 15 %	Over 15 No.	Over 15 %
Normal	100	100	95	95	5	5	0	0	0	0
Hospital routine	100	100	86	86	12	12	0	0	2	2
Infected (not ill)	11	98	92	94	6	6	0	0	0	0
Acute hepatitis	35	45	3	7	0	0	5	11	37	82
Convalescent hepatitis	32	51	31	60	9	18	7	14	4	8
Cirrhosis (all types)	25	25	14	56	4	16	4	16	3	12
Tumors involving liver	11	11	1	9	5	45	4	36	1	9
Hemolytic and obstructive jaundice	7	7	4	57	3	43	0	0	0	0

Incubation at 5° C. for 18 to 20 hours. Scores calculated on the basis of a 7 tube titration test beginning with a serum dilution of 1 to 4.

A total of 45 sera from 35 patients with infectious hepatitis of 14 or less days duration was examined by the titration procedure (Table 7). Five samples reacted with a score of 11 to 15 and were, therefore, classed as weakly positive. Strong flocculation was exhibited by 37 sera each with cumulative scores exceeding 15; 3 specimens, none of which appeared grossly icteric, scored 5 or less; 2 of these were obtained from 1 patient on the 10th and 14th days of illness; the other was drawn on the 5th day of disease. From the above data it was concluded that the titration method was sufficiently sensitive to detect more than 90% of infectious hepatitis in the acute phase.

The sera from convalescent hepatitis, cirrhosis and tumors involving the liver parenchyma showed intermediate types of reactions which varied with the ability of

tenfold dilutions of serum were more useful for the interpretation of results than the single tube test. This contention, however, was handicapped by the lack of normal serum controls as pointed out shortly thereafter by Mirsky and von Brecht.[10] They found that 0.2 ml. of serum in the standard test gave satisfactory negative results but that reduction to 0.1 ml. or less resulted in flocculation in practically all normal specimens. For this reason they did not consider the Bruger procedure to be a valid one for the detection of alterations in hepatic function. We have obobtained similar results with the Hanger type of antigen in titration tests carried out at 5° C. Such false positive reactions with normal sera may perhaps be due to dilution of the inhibiting albumin component as suggested by the data of Moore *et al.*[11] in their studies of the mechanism

responsible for the cephalin-cholesterol flocculation. Modification of the method of preparation of the antigen emulsion has resulted in a reduction of flocculation in all zones of dilution of normal serum, thus permitting the use of a titration technique. A study of flocculation over a range of serum dilutions has the additional advantage of minimizing errors frequently associated with single tube tests.

With the modified antigen it is no longer necessary to limit tests to freshly drawn sera. Specimens may be stored and examined under the same conditions with a single lot of standardized cephalin cholesterol emulsion. Furthermore, in equivocal cases the sera may be reëxamined for comparison with sera obtained later in the clinical course. The elimination of interfering photosensitivity and error due to prolonged standing of serum in the diluted form represents further advantages of the new procedure. The selection of 5° C. as the temperature for incubation was not entirely based on the evidence presented in Table 3. In experiments not shown in the protocols certain acute hepatitic sera flocculated the antigen poorly at 21° C. but strongly at 5° C. Other factors which influenced the decision to use ice-box incubation were the variability in room temperature throughout the year and the possibility of bacterial contamination. The data suggest a number of factors in the flocculation test which remain to be studied. The variation among different commercial lots of antigen as seen in Table 5 point to the necessity of introducing proper standardization procedures. The selective elimination of flocculation with normal sera by heating to 56° C. appears possible from the evidence in Table 4.

Some acute sera from patients in whom a diagnosis of infectious hepatitis had been made failed to react at all, a finding which did not agree with the clinical impression. Negative reactions under these conditions may either indicate an error in diagnosis or mild parenchymatous disease. In the latter instances and during convalescence the fluctuations noted in the titration test may have greater prognostic significance than the limited variations which can be recorded with the standard Hanger procedure.[2,3,5,6,9,14,16,17] This opinion is illustrated by the data presented in Table 6 where the sera of Subjects 2 and 3 showed a sharp decline in cumulative score while the Hanger test remained 4+; with Patient 4, the prolonged illness was accompanied by a continued abnormality in the cumulative score, after the Hanger test had become negative.

Summary. 1. Confirmation is reported of observations by others that cephalin cholesterol emulsions prepared by the Hanger technique are flocculated by normal sera which have been diluted and exposed to light or permitted to age.

2. A modified method of preparing a cephalin cholesterol emulsion from the stock etherized solution is described and some factors influencing the sensitivity of this antigen are presented.

3. The modified emulsion is not flocculated with normal sera which have been exposed to light, diluted, or aged. It does, however, flocculate satisfactorily in the presence of sera from patients with parenchymatous liver diseases.

4. Tests made with serial dilutions of serum and utilizing the modified emulsion permits more accurate interpretation of the flocculation pattern in liver disease than does the procedure of Hanger.

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THE EFFECT OF PROSTIGMINE (NEOSTIGMINE) ON THE MUSCLE SPASM OF RHEUMATOID ARTHRITIS*

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Prostigmine has been advocated in recent years for the relief of muscle spasm in anterior poliomyelitis and in a variety of diseased states of the central nervous system.[2,3,5,4,9,13] More recent work has suggested that prostigmine might be of value for the relief of muscle spasm associated with arthritic conditions.[8,17] The purpose of the present study was to evaluate the effect of prostigmine on the muscle spasm of rheumatoid arthritis.

Prostigmine (neostigmine) is a synthetic alkaloid belonging to the physostigmine group of drugs. Its fundamental pharmacologic action is thought to be due primarily to its inactivation of the enzyme cholinesterase, thus diminishing the breakdown of acetylcholine throughout the body. Acetylcholine is the chemical mediator of nerve impulses to the end-organs of the voluntary nervous system and of the cholinergic division of the autonomic nervous system.[1] Atropine can block the cholinergic effects of prostigmine on the vagal end-organs and the sweat glands, but does not have this effect on the myoneural junction of skeletal muscle.[7] Under the influence of toxic amounts of prostigmine, fasciculations of skeletal muscle occur in spite of atropinization. Thus it appears that prostigmine lowers the threshold of response of skeletal muscle, making it more irritable by preserving acetylcholine at the myoneural junctions, and to this is attributed its well-known therapeutic value in myasthenia gravis. The action of prostigmine on the central nervous system is less well understood. If, as postulated by Loewi,[11] acetylcholine acts as the chemical transmitter of nerve impulses in the synapses of the brain and spinal cord, prostigmine might well be expected to be an excitant of the central nervous system and to increase the ease of transmission of nerve impulses through the central nervous system. However, to the contrary, most observers[10,12,14,15,16] have found that prostigmine administered either intrathecally or intravenously depresses the spinal reflexes in mammals and man. The physiologic mechanism by which this depression of the spinal reflex arc is brought about is not clearly understood. However, it has been shown that while small doses of acetylcholine stimulate nerve synapses, larger doses paralyze them.[11] It is probable that the action of prostigmine on the central nervous system is due to its acetylcholine sparing effect. Atropine does not block the action of prostigmine on the spinal cord.[15]

Prostigmine was first suggested for use in combating muscle spasm in anterior poliomyelitis by Kabat and Knapp[9] in 1943. They treated 20 cases of subacute poliomyelitis with a combination of sub-

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TABLE 1.—EFFECTS OF PROSTIGMINE THERAPY IN CASES OF RHEUMATOID ARTHRITIS

			Prostigmine bromide											
			15 mg., t.i.d.			30 mg., t.i.d.			45 mg., t.i.d.			Benefit (total)	
Case	Involved joints	Motion before	Days	Motion	Effects	Days	Motion	Effects	Days	Motion	Effects	Motion	Effect
1	L. knee	110°	4	113°	Relaxation very sl.	3	110°	Cramps	1	110°	Abd. cramps	+ 3°	None
2	R. shoulder—abduction	110°	5	120°	Subject. more relaxed; no toxic effects noted	8	115°	Joint movement feels freer; no toxic effects noted	1	120°	Abd. cramps even with extra doses of atropine	+10°	More relaxed on drug; benefit cont. after
	R. elbow	80°	5	100°		8	110°		1	110°		+30°	
	R. wrist—flex.-ext.	60°	5	70°		8	70°		1	70°		+10°	
	R. hip—flex. ext.	100°	5	110°		8	125°		1	130°		+30°	
	L. hip—flex.-ext.	100°	5	125°		8	135°		1	145°		+45°	
	L. knee	80°	5	85°		8	85°		1	85°		+ 5°	
3	R. knee	100°	7	95°	Sl. relaxation only and sl. cramps	..	..	Stopped after 1 dose; abdominal cramps	..	..	....	− 5°	Sl. relaxation temporary
	L. knee	75°	7	85°		..	..		..	..	....	+10°	
	L. elbow	80°	7	75°		..	..		..	..	....	− 5°	
4	R. knee	115°	3	115°	Relaxation mild	14	120°	Relaxation mild	..	..	....	+ 5°	Relaxation sl.
	L. knee	115°	3	115°		14	120°		..	..	....	+ 5°	
5	R. elbow	60°	3	77°	None	..	..	Discont. after 1 dose; cramps	..	..	....	+17°	None
	R. wrist—flex. ext.	52°	3	37°	None	..	..		..	..	....	−15°	None
	supin.-pron.	60°	..	45°		..	..		..	..	....	−15°	
	L. wrist—flex.-ext.	84°	3	92°	None	..	..		..	..	....	+ 8°	None
	supin.-pron.	90°		105°		..	..		..	..	....	+15°	
6	R. knee	100°	3	113°	Sl. relaxation	6	100°	Abd. cramps	..	..	....	+10°	None
7	L. shoulder—abduction	60°	8	60°	None	..	..	....	..	..	....	0°	None
	flex.-ext.	150°	8	145°	None	..	..	....	..	..	....	− 5°	None
	rotation	135°	8	125°	None	..	..	....	..	..	....	−10°	None
8	L. hip—flex.-ext.	100°	6	100°	None	7	110°	Abd. cramps mod.	3	105°	Abd. cramps severe	+ 5°	None
	R. hip—flex.-ext.	50°	6	50°	None	7	50°		3	50°		0°	None
	rotation	20°	6	20°		7	20°		3	20°		0°	
9	Spine—flexion: fingers to floor	9″	9	6″	None	4	6″	None	3	8″	None	+ 1″	None
	S.L.R.—R.	105°	9	105°	None	4	105°		3	105°		0°	
	S.L.R.—L.	120°	9	110°	None	4	110°	None	3	110°	None	+10°	None
10	Spine—flexion: fingers to floor	15″	3	15″	None	6	14″	None	..	..	....	+ 1″	None
	S.L.R.—R.	125°	3	130°	None	6	120°	None	..	.	....	+ 5°	None
	S.L.R.—L.	125°	3	125°	None	6	125°	None	..	..	....	0°	None
12	Spine—flexion: fingers to floor	15″	3	15″	None	6	9″	None	..	..	....	+ 6″	None
	S.L.R.—R.	140°	3	145°	None	6	145°	None	..	..	....	− 5°	None
	S.L.R.—L.	130°	3	135°	None	6	130°	None	..	..	....	− 5°	None
13	Spine—flexion: fingers to floor	15″	3	13″	None	6	13½″	None	..	..	....	+1½″	None
	S.L.R.—R.	120°	3	125°	None	6	120°	None	..	..	....	0°	None
	S.L.R.—L.	125°	3	125°	None	6	130°	None	..	..	....	− 5°	None
14	Neck rotation	90°	3	100°	Sl. relaxation	7	100°	None	3	100°	Abd. cramps; more back pain	+10°	None
	Spine—flexion: fingers to floor	19″	3	22″	Back more painful	7	24″	Stiffer back and hips	3	22″		− 3″	None
	S.L.R.—R.	120°	3	115°		7	125°		3	130°	Muscle twitch,	−10°	None
	S.L.R.—L.	130°	3	125°		7	120°		3	130°		−10°	None

	floor													
	S.L.R.—R.	120°	3	125°	None	6	120°	None	..	..	....	+1½″	None	
	S.L.R.—L.	125°	3	125°	None	6	130°	None	..	..	....	0°	None	
												− 5°	None	
14	Neck rotation	90°	3	100°	Sl. relaxation	7	100°	None	3	100°	Abd. cramps;	+10°	None	
	Spine—flexion: fingers to floor	19″	3	22″	Back more painful	7	24″	Stiffer back and hips	3	22″	more back pain	− 3″	None	
	S.L.R.—R.	120°	3	115°		7	125°		3	130°	Muscle twitch.	−10°	None	
	S.L.R.—L.	130°	3	125°		7	130°		3	130°		0°	None	
15	Spine—flexion: fingers to floor	22″	3	23″	None	6	22″	None	2	23½″	Abd. cramps	−1½″	None	
	Chest expansion	1¼″	3	1″	None	6	1¼″	None	2	1½″		+ ¼″	None	
	S.L.R.—R.	145°	3	130°	None	6	140°	None	2	130°		+15°	None	
	S.L.R.—L.	145°	3	120°	None	6	140°	None	2	160°		−15°	None	
16	Spine—flexion: fingers to floor	12″	3	12″	None	2	13″	None	..	..	. .	− 1″	None	
	S.L.R.—R.	125°	3	125°	None	2	130°	None	..	..	....	− 5°	None	
	S.L.R.—L.	115°	3	120°	None	2	120°	None	..	..	....	− 5°	None	

TABLE 2. EFFECT OF A SINGLE DOSE OF PROSTIGMINE BROMIDE ON SPASM OF RHEUMATOID ARTHRITIS

45 mg. prostigmine bromide and 0.6 mg. atropine sulfate orally

Case	Involved joints	Motion before treatment	1 hour after dose		2 hours after dose		4 hours after dose		Benefit
			Motion	Effects	Motion	Effects	Motion	Effects	
17	L. shoulder—abduction	60°	55°	Sl. vertigo; no relaxation	55°	None	60°	None	None
	flexion	30°	35°		40°		40°		+10°
	R. shoulder—abduction	60°	55°		50°		55°		− 5°
	flexion	40°	35°		40°		40°		None
18	R. elbow	115°	110°	No effect	115°	Muscle twitching	110°	Muscle twitching	− 5°
19	R. hip—flexion	55°	60°	No effect; extra 0.6 mg. atropine given	60°	No effect	60°	Abdominal cramps mod.	+ 5°
	abduction	50°	50°		40°		40°		−10°
	R. knee	120°	120°		120°		120°		None
20	L. ankle—invers.-evers.	10°	10°	No effect	10°	Mild muscle twitching	10°	No effect	None
	flex.-ext.	40°	45°		40°		40°		
21	L. elbow	70°	70°	Sweating; reflexes more active	70°	Decr. sweating; more relaxed	70°	Abdominal cramps mod.	None
	L. knee	125°	120°		125°		125°		None
	R. knee	95°	95°		97°		95°		None
22	L. knee	90°	85°	Severe twitch.; more atropine given, 1 mg.	85°	Severe twitch., cramps and nausea	90°	Persistent twitch.; reflexes increased	None
23	L. wrist—flex.-ext.	20°	20°	Reflexes more active; given atropine, 6 mg.	20°	Abdominal cramps and muscle twitching	20°	Occas. muscle twitching	None
	R. wrist—flex.-ext.	30°	30°		30°		30°		None
	Neck—rotation	60°	60°	....	50°	....	60°	....	None
	flex.-ext.	70°	60°	....	60°	....	65°	....	− 5°
	lat. deviation	40°	50°	....	50°	....	45°	....	+ 5°

cutaneous and oral prostigmine and noted in all cases a significantly increased range of motion of the afflicted limbs and in some instances a decrease in deformities by relaxation of muscle hypertonus. They theorized that the beneficial response obtained was due to the anticholinesterase effect of prostigmine on the synapses of the internuncial neurons. These neurons they take to be functionally impaired in poliomyelitis. Other investigators[2,3,4] have made similar clinical observations with prostigmine in acute and subacute anterior poliomyelitis, though in their cases the relaxation of muscle spasticity was much less dramatic than in the series of Kabat and Knapp.[9] Further studies in anterior poliomyelitis were carried out by Watkins and Brazier[16] who quantitatively measured the electrical discharges from the spastic muscles before and after the intramuscular injection of prostigmine. They were unable to detect changes after use of prostigmine which they could accept as of significant degree to indicate a specific effect on the muscle spasm.

Trommer and Cohen[17] applied the principles of Kabat and Knapp's work in poliomyelitis to various joint diseases with associated muscle spasm. They reported that, in 13 of their 19 cases treated with prostigmine (including some with rheumatoid arthritis), the muscle spasm was diminished. Kabat[8] has also reported that prostigmine reduced muscle spasm in patients having various types of joint and muscle disease. Thus prostigmine has been put forth by these authors as beneficial in releasing the muscle spasm of rheumatoid arthritis and allied conditions.

Method. Prostigmine was administered to 23 patients with active rheumatoid arthritis. All patients were hospitalized during the period of prostigmine therapy and under the constant surveillance of the authors. Of the cases, 13 were rheumatoid arthritis with various peripheral joints involved, 8 cases were rheumatoid spondylitis with involvement of the sacro-iliac joints and spine, and 2 cases had involvement of both the spine and sacro-iliac joints as well as peripheral joints. All cases were relatively early in the course of their present attack. Activity in each instance was manifested subjectively by malaise, pain and stiffness of the affected joints, and objectively by weight loss, swollen tender joints with spasm of the surrounding muscles, and elevated erythrocyte sedimentation rates. The patients with spondylitis had Roentgen ray abnormalities in the sacro-iliac joints, decreased chest expansion, limitation of straight leg raising (S.L.R.), marked spasm of the erector spinæ muscles, and limitation of motion of all or part of the spine. None of the cases had ankylosed joints or advanced contractures. A goniometer was employed to determine in degrees the maximum range of motion of the affected joints before prostigmine was administered and at various subsequent intervals. In patients having spondylitis, goniometric determinations were made of the angle of straight leg raising (S.L.R.). Flexion of the spine was estimated by measuring the distance from the finger-tips to the floor with the patient flexing the spine with the knees in extension. All patients had been receiving physical therapy for varying periods and this was continued uninterruptedly during the present study.

In each of the first 16 cases studied (Table 1) 0.5 mg. prostigmine methyl sulfate and 0.6 mg. atropine sulfate were injected subcutaneously. Three hours after the initial dose, prostigmine bromide was administered orally in doses, which, if tolerated, increased from 15 to 30 to 45 mg. 3 times each day. Atropine sulfate (0.6 mg.) was given with each dose of prostigmine. Determinations of joint mobility were made prior to each increase in the dose of prostigmine and on terminating therapy.

Seven cases were later studied (Table 2) to determine the immediate effect of a single large dose of prostigmine on muscle spasm. Each of these patients was given 45 mg. of prostigmine and 0.6 mg. atropine sulfate orally. Observations including

measurements of range of joint motion were made at 1, 2 and 4 hours after administration.

Discussion. As shown in Table 1, only 2 cases showed an increase in range of motion of more than 10° following the use of prostigmine. Our experience and that of others[5] is that goniometric measurements are reliable only to within 10°. Because of minor errors in measurements and the known daily variation in intensity of muscle spasm in rheumatoid arthritis, we think that changes of 10° or less are not significant. Of the 2 cases which did show more than 10° increased motion in some of the affected joints, 1 (Case 5) showed a concomitant decreased range of motion of 15° in 1 of the 3 joints studied. The other case (Case 2) showed a significant increased range of motion in all 6 joints measured. However, it later became evident that this patient's arthritis was going into a spontaneous remission at the time these studies were undertaken. Some degree of subjective improvement was noted by 6 patients (Cases 1, 2, 3, 4, 6, 14) at some time during the study, but in only 1 of these (Case 2) was there significant objective evidence of a decrease in muscle spasm.

Three cases could not tolerate more than 15 mg. of prostigmine 3 times a day in spite of atropinization. Seven cases could take only 30 mg. 3 times a day, and 6 cases 45 mg. 3 times a day. There was no correlation between the amount of drug tolerated and the degree of benefit noted.

None of the 7 patients given a single massive dose of prostigmine showed any significant objective release of muscle spasm as manifested by increased range of motion in the affected joints, and none experienced any subjective improvement. In fact, 6 of the 7 cases developed unpleasant toxic reactions. Four showed generalized muscular fasciculations and 2 had abdominal cramps throughout the period of observation.

The disparity between our findings with prostigmine in rheumatoid arthritis and those of other observers in anterior poliomyelitis possibly may be attributed to the different origin of the muscle spasm in the 2 conditions. In anterior poliomyelitis the muscle spasm is a complex phenomenon due to inflammation in the spinal cord with secondary changes in the muscles themselves. In rheumatoid arthritis, on the other hand, the spasm is due to prolonged guarding against movement of a painful joint, and also to inflammatory changes in the muscles, fibrous tissue and peripheral nerves as described by Freund, Steiner, Lichtentritt and Price.[5,6] The central action of prostigmine, such as it may be, is apparently insufficient to affect significantly the muscle spasm of rheumatoid arthritis. Previous observations have confirmed that the action of prostigmine on skeletal muscle is that of an excitant, and this explains the muscular fasciculations seen in so many of our cases. Although this theory might explain the failure of prostigmine to control spasticity in rheumatoid arthritis, even though effective in diseases such as poliomyelitis, we cannot explain the obvious difference between our results and those reported by Trommer and Cohen,[17] and Kabat[8] in rheumatic conditions.

From our data we conclude that prostigmine induces no significant relaxation of muscle spasm in rheumatoid arthritis. The slight variation in range of motion noted in our cases can be accounted for by the daily variations caused by changes in weather, fatigue, or other influences which so commonly make the rheumatic patient better on some days and worse on others. We have avoided emphasis on subjective benefit, as many rheumatic sufferers are highly suggestible, often expressing some relief from placebos alone. Objective findings are little influenced by the receptiveness of the individual to a new form of therapy, and so are more reliable. In our opinion, a potent drug such as prostigmine should yield definite benefit to justify its continued use in rheumatoid arthritis. This we have not found.

Summary. 1. Prostigmine in doses up to the limit of tolerance was administered to 23 patients with rheumatoid arthritis in an attempt to relieve the associated muscle spasm.

2. In no case was a satisfactory therapeutic response obtained. Only 2 cases showed any appreciable decrease in muscle spasm, and even in 1 of these the effect was inconsistent, as the spasm about other affected joints coincidentally increased. The second case had already started into spontaneous remission.

3. Prostigmine caused unpleasant and often alarming reactions in 14 of the 23 cases studied.

4. We do not recommend prostigmine in the treatment of rheumatoid arthritis.

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MELITURIA IN HEALTHY AMERICAN MEN WITH SPECIAL REFERENCE TO TRANSITORY GLYCOSURIA

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Whenever any large group of individuals presumably healthy are surveyed by urine analysis, a certain small percentage will be found to be excreting reducing substances at the hour of the survey. Opportunities to discover and study such questionably abnormal individuals have become more numerous in the past few years, as a direct consequence of the fitness examinations being given to millions of young men and women prior to their entry into the fighting forces. The present paper summarizes the experiences and follow-up studies made on 77,293 consecutive male enrollees received at a large Maritime Service Training Station. When screened by urine analysis within the 1st day or 2 of reporting for duty, 324 (0.42%) gave a positive reaction to the Benedict test for sugar in the urine.

Few reports on the incidence of melituria in recruits for this war have as yet appeared. Peel and Peel[9] studied some 115 cases of glycosuria encountered in an unspecified number of men called up for service with the British Army. Of these, 43 were classed as clear-cut diabetes, 42 as borderline diabetes, and 30 as renal glycosuria. Blotner and Hyde[2] recounted their experience with 45,650 consecutive military inductees from the Boston area. Melituria was found in 367 (0.8%), divided as follows: diabetes mellitus, 208 (0.46%); transient glycosuria, 126 (0.28%) renal glycosuria, 3 (0.07%). Spellberg and Leff[11] reported on 32,033 similar inductees seen at New Orleans; 37 instances of glycosuria were found, of whom only 9 were diagnosed as having diabetes mellitus. The etiology of the sugar in the urine of the non-diabetics was not investigated. None of these reports gives information as to whether the population of eligible males from which the inductees were drawn had been screened by prior physical examination including urine analysis.[13b]

During the war years the number of men volunteering for Maritime Service training was always greater than the need. It was the custom, therefore, to reject summarily, at the enrolling offices, all applicants found with sugar in the urine. Further studies were made only when evidence was submitted from outside sources that the melituria was benign, or when an enrolling physician chanced to take a special interest in some applicant. The incidence of the varieties of melituria in the series here reported is therefore not representative for the population as a whole. The findings portray conditions existent in a selected group of American adult males, adjudged to be in good health by a physical examination received within the preceding few weeks.

For strict accuracy no individual with melituria should be diagnosed as having glycosuria or dextrosuria until the excreted sugar has been actually identified as dextrose. Such identification can be carried out with reasonable reliability by any clinical laboratory, though incontrovertible recognition requires skill, time, and much special equipment. Dextrose and also levulose become completely destroyed on incubation with baker's yeast at 30° C. for 2 to 6 hours, whereas galactose and pentose resist fermentation under these circumstances. Levulosuria (fructosuria) is so rare that any yeast-fermentable reducing substance found in urine may be held to be dextrose without further investigation, unless the attendant circumstances are peculiar.

"Pseudomelituria" may be met with at times, due to the presence in the urine

of reducing substances other than sugars. The best way to avoid such false reactions is by correct application of the test procedures. When properly carried out in the recommended proportions Benedict's test is rather highly specific.[10] With no more than 2 minutes boiling over a flame, or 3 minutes in a water-bath, this reagent has a threshold sensitivity of about 50 mg. of sugar per 100 ml. of urine (0.05%). The false positives it gives are usually traceable to more prolonged exposure to heat. It is much less prone than tests of the Fehling or Trommer type to give positive reactions in the absence of a true sugar.

Classification and Criteria. 1. Transitory Melituria. (*a*) *Alimentary.* Fruits or fruit juices in excess evoke sucrosuria or glucosuria; candy or toast may induce dextrosuria, maltosuria, or dextrinuria; excess lactose or galactose results in lactosuria or galactosuria. Overflow meliturias of this sort, of dietary origin, are transitory and harmless. Of these sugars the only ones which will ordinarily be picked up by Benedict's test are maltose and dextrose.

(*b*) *Toxic.* Patients ill with sundry disorders sometimes excrete dextrose in the urine. This has been reported in pregnancy, furunculosis, lead poisoning, cerebral conditions, hyperthyroidism, hyperpituitarism, and febrile states.

(*c*) *Emotional.* High emotional strain can excite a temporary dextrosuria. The current physiologic explanation of this phenomenon, based on Cannon's cat experiments, is that under strong emotion, such as fright, epinephrin becomes secreted in augmented degree.[4,6] The epinephrin mobilizes hepatic glycogen and transitorily raises the blood sugar level above the renal threshold.

2. Renal Glycosuria. This condition is often familial. The excretion of dextrose is chronic, continuous, non-progressive, and harmless. The quantity of dextrose excreted differs from patient to patient; it may fluctuate with variations in carbohydrate intake but in the more marked instances is largely independent of diet. Reduction of blood sugar by insulin may transiently inhibit the excretion. In accord with Marble's[7] recommendation, only those individuals who excrete dextrose continuously through day and night should be diagnosed as having renal glycosuria.

3. Low Renal Threshold. This diagnosis is reserved for those non-diabetic individuals who have negative urine when fasting but who excrete sugar following a meal containing carbohydrate. Mosenthal and Ashe[8] have described how the blood threshold levels for dextrose excretion may range between 100 and 160 mg. per 100 ml. The latter figure is commonly taken as the lower limit of normal renal threshold for dextrose.

In both renal glycosuria and low renal threshold there are no symptoms and no hyperglycemia, either during fasting or after a meal. The dextrose tolerance test reveals a normal or unusually flat curve. Treatment is not needed. Every newly discovered case should be observed medically for some years, inasmuch as rare patients with beginning diabetes mellitus may simulate these conditions.

4. Diabetes Mellitus. The diagnostic tool relied upon most for establishing the presence or absence of diabetes is the dextrose tolerance test, in one or another of its variant forms. To ascertain the approximate renal threshold urine samples are tested at the same times that blood specimens are taken. With the 1 dose-3 hour test, the fasting blood sugar is first determined, 100 gm. of dextrose are given orally, and specimens of venous blood collected at $\frac{1}{2}$, 1, 2 and 3 hour intervals. The standards taken for top normal response are: a fasting reading of less than about 0.12 gm. sugar per 100 ml. of blood, a $\frac{1}{2}$ hour value of less than about 0.18 gm. per 100 ml., and a return to 0.12 mg. by 2 hours. With the Exton-Rose 2 dose-1 hour test the fasting blood sugar is first determined, 50 gm. of dextrose are given orally, a second blood specimen is taken after 30 minutes, a

second dose of 50 gm. dextrose given, and a final blood specimen collected 30 minutes later. The standards taken for top normal response are: a fasting level of less than about 0.12 gm. per 100 ml., and ½ and 1 hour blood sugar values no higher than 0.16 to 0.18 gm.[5] Prior to performing either of these tests—and borderline cases receive both—300 gm. of dextrose solution in excess of the normal diet is usually fed for 2 or 3 days, in order to stimulate the insulin-producing mechanisms. Thus are avoided the confusing results so often obtained when the antecedent diet has been low in carbohydrates or inadequate in calories.

Some physicians are content to exclude the diagnosis of diabetes mellitus in suspects whose arrival specimens contained sugar, if additional specimens, collected postprandially, are sugar-free. Without doubt this practice rules out well-established diabetes (if untreated) but mild or incipient cases can slip through a screen consisting solely of repeated urine tests. Such incipient cases (the so-called latent or potential or "pre-" diabetics) can be sugar-free after a fast and in the early morning, but will excrete sugar after ingestion of sweets or a large meal. The dextrose tolerance test is a much more sensitive and reliable procedure. In otherwise healthy individuals, under appropriate conditions, "negative reactions to tolerance tests almost always can be accepted with assurance as evidence of absence of diabetes." (Wilder.[12])

5. Congenital Non-dextrosic Melituria. Pentosuria, fructosuria, galactosuria and sucrosuria are recognized hereditary abnormalities. Small amounts of the respective sugars are excreted more or less continuously into the urine while the dextrose metabolism remains entirely normal.[13a] Lactosuria does not occur in adult males.

6. Undiagnosed. In this group we have placed all who were transferred to other stations or who disenrolled, voluntarily or otherwise, before diagnostic studies were complete.

Subjects and Methods. Between March 1943 and April 1945, 77,293 consecutive male trainees were admitted to the Sheepshead Bay Training Station of the U. S. Maritime Service. These men were nearly all American citizens, and were recruited in the northeastern quarter of the United States. At the time of reporting they were free from obvious illnesses apart from occasional upper respiratory tract infections or other minor acute disabilities. All had satisfactorily passed physical examinations and presumably had sugar-free urine when enrolled at regional offices in the month prior. Their urine specimens were usually collected for test on the day following arrival at the training station, in mid-morning or mid-afternoon, between 1 and 3 hours after a meal.

The examination period found most of the men fatigued and emotionally upset. Their home ties and business affiliations had just been broken, and a new pattern of living was about to begin. Many had been travelling long distances, on uncomfortable stuffy trains with poor facilities for eating and sleeping. On arrival they had been ushered into an impersonal and bewildering military routine. They were now being taken to the medical department for a physical going-over, with the foreknowledge that painful inoculations would immediately follow.

The urine specimens were tested for sugar with Benedict's qualitative reagent. The tubes were placed in a boiling water bath for 3 minutes exactly, and readings made immediately afterward. Positive specimens were next subjected to Benedict's quantitative test, and the content of reducing substances expressed in terms of dextrose. With every subject having persisting melituria, and with 65 successive individuals having transitory melituria, the sugar in the urine was identified as dextrose in every case by yeast fermentation. It has therefore been assumed that with all subjects in this series the excreted reducing substance has been dextrose.

All men with positive urine findings were called back by the medical department for a clinical history, further urine analyses, and blood studies in most instances.

Results and Discussion. Of the total of 77,293 arriving trainees, 324 (0.42%) exhibited melituria. On diagnostic study

the great majority of these were found to have melituria of harmless nature. Only 21 (7.7%) of the positive cases had diabetes (Table 1).

The age distributions are given in Table 2 and the breakdown in terms of concentration of sugar in the urine in Table 4. There were no indications with any subject that the excreted sugar was other than dextrose.

known mechanisms active in "emotional" glycosuria.

Information is not available as to the state of carbohydrate "saturation" of the men at the time of their arrival; but borderline responses were frequently encountered in their first dextrose tolerance tests. The fasting blood sugar levels were frequently in the high normal zone, or an unusually high peak appeared in the

TABLE 1.—DIAGNOSTIC BREAKDOWN OF THE 324 INSTANCES OF MELITURIA FOUND AMONG 77,293 TRAINEES

Type	No.	Absolute %	Comparative %
1. Transitory	225	0.29	82.7
2. Low renal threshold	19	0.02	7.0
3. Renal glycosuria	7	0.01	2.6
4. Diabetes mellitus	21	0.03	7.7
5. Undiagnosed (disenrollments, transfers, etc.)	52	0.07	
Total	324	0.42	100.0

TABLE 2.—INCIDENCE AND PERCENTAGE DISTRIBUTION OF MELITURIA ACCORDING TO AGE GROUPS

Age groups (yrs.)	No. enrollees	Transitory	Low renal threshold	Renal glycosuria	Diabetes mellitus	Undiagnosed	Totals
16–20	44,586	88 (0.20%)	9 (0.02%)	4 (0.01%)	9 (0.02%)	15 (0.03%)	125 (0.28%)
21–25	13,783	28 (0.20%)	2 (0.02%)	...	3 (0.02%)	11 (0.08%)	44 (0.32%)
26–30	10,911	40 (0.36%)	1 (0.01%)	1 (0.01%)	4 (0.04%)	11 (0.10%)	57 (0.52%)
31–35	5,681	45 (0.79%)	4 (0.07%)	2 (0.04%)	1 (0.02%)	8 (0.14%)	60 (1.06%)
36 and over	2,332	24 (1.03%)	3 (0.13%)	...	4 (0.17%)	7 (0.30%)	38 (1.63%)
Totals	77,293	225 (0.29%)	19 (0.02%)	7 (0.01%)	21 (0.03%)	52 (0.07%)	324 (0.42%)

In 82.7% the diagnosis of transitory melituria was arrived at. This large group consisted of men who exhibited reducing substances in the urine but once (or in 34 instances, twice), and in whom neither a diabetically disturbed carbohydrate metabolism nor a permanently lowered renal threshold were demonstrable.

Re-study of this group after a few years, were this possible, might uncover a certain percentage having more advanced disturbances sufficient to admit the recognition of diabetes mellitus.

Many of those whose urines exhibited reducing substances on 2 occasions before becoming consistently negative may have had unrecognized low renal thresholds. Possibly a number of the men had indulged in candy bars or high carbohydrate foods a few hours prior to their voiding of the first specimen. In others the excitement and novelty attendant upon the 1st day of training may have stimulated the unfirst specimen collected after ingestion of the test dose of carbohydrate, or the curve, otherwise normal, failed to drop to the fasting level until 2 hours had passed. On repetition a few days later, after forcing of carbohydrates, these peculiarities of the blood sugar curve would no longer be found.

The rise in incidence of positive reactions above 25 years of age (Table 2) runs more or less parallel to the familiar age trend for the incidence of diabetes mellitus. This suggests that transitory melituria is an early expression of a lack of flexibility in internal carbohydrate economy. It is of interest that no mention of a possible relationship between transitory melituria and advance in age is to be found in the recent reviews and textbooks consulted as references.

For this group one can say only that at the time and under the circumstances of the screening survey about 1 of every

289 healthy enrollees undergoing examination was found to be temporarily excreting a reducing substance, presumptively dextrose. The possible pathogenesis is discussed further in a later section.

With the 7 cases diagnosed renal glycosuria all urine specimens contained sugar, including those secured when the blood levels were as low as 60 to 80 mg. per 100 ml. Several had positive family histories of the same condition, and 2 were already aware of its existence in themselves. With 4 the dextrose tolerance curves were strikingly "flat." The quantity of sugar excreted by each patient was of a more or less constant order of magnitude in hour-to-hour and day-to-day sampling, the average concentrations being, respectively, 0.5, 0.7, 0.7, 0.8, 1, 1.2, and 1.3 mg. per 100 ml. of urine.

whenever an arbitrary line is drawn, borderline findings are bound to be met. With controversial cases the interpretation and handling fluctuated with the clinical acumen and professional interest of the attending physicians.

Of the 21 patients with diabetes mellitus, 17 were free from symptoms and ignorant of their illness. In 2 men the urine contained ketone bodies as well as dextrose: 1 confessed to having taken insulin for 5 years, the other for 6 years. The 2 others had been rejected recently by the army because of early diabetes. All 4 had hoped to go through the training course without the existence of the diabetes being discovered. Not included in this tabulation are some additional cases of diabetes which came to light during the course of training, men whose urine examinations has been negative on arrival.

TABLE 3.—APPROXIMATE DISTRIBUTION OF CRITICAL BLOOD SUGAR VALUES IN 19 CASES HAVING LOW RENAL THRESHOLD (VENOUS BLOOD)

Renal threshold blood sugar (mg. per 100 ml.)	No. cases	Renal threshold blood sugar (mg. per 100 ml.)	No. cases
91–100	2	131–140	3
101–110	2	141–150	6
111–120	0	151–160	4
121–130	2		

In the low renal threshold group exact identification of the urine sugar was not always possible, but non-dextrosic melituria was ruled out by virtue of the fact that these individuals had sugar-free urine when fasting. From the results of the dextrose tolerance tests an effort has been made to estimate the levels of blood sugar at which sugar appeared in the urine. This is not highly exact as a method for determining renal thresholds, but a summary of the observations does give a rough picture of the scattered distribution (Table 3).

The equivocal instances of diabetes mellitus were given the benefit of the doubt. Only those whose carbohydrate metabolism was disturbed beyond question were diagnosed as having diabetes and given medical discharges. Family history was taken into consideration in arriving at the diagnoses. To be sure,

No instance of any of the varieties of congenital non-dextrosic melituria were met with among this large series of normal men. This is not surprising since the occurrence rate of pentosuria, which is the most common kind, has been estimated by Blatherwick[1] as 1 in 50,000. Bock[3] estimated the prevalence of fructosuria as less than 1 in 100,000.

For the undiagnosed cases the distribution of age and sugar concentration ran closely parallel to those for the total series, indicating the random unbiased character of this group (Tables 2 and 4).

Pathogenesis of Transitory Dextrosuria. Fourteen men with transitory dextrosuria were subjected to more intensive laboratory studies. It was first established by fermentation tests that the reducing sugar they excreted was dextrose.

The hypothesis was explored that a state of hyperglycemia evoked by strong emotion existed at the time of the test. Certainly the circumstances under which the urine specimens were being secured were conducive to apprehension and emotional excitement. Blood specimens for assay were accordingly drawn as soon as the urine was found positive—never more than 15 to 30 minutes after voiding. The blood was collected on the medical examining line, just ahead of the room where inoculations were being given. *No unusually high blood sugar levels were discovered under these emotion-provoking circumstances.* With 10 specimens the sugar content was between 73 and 100 mg. per 100 ml.; in the remaining 4 it fell between 120 and 148 mg. per 100 ml. This absence of striking rise in blood sugar in humans undergoing emotional reactions is in line with the experience of other workers who have studied this problem.[6]

Subsequent tests showed that only 1 of these 14 subjects had a renal threshold below 160 mg. per 100 ml. blood level. This individual "spilled over" at 133 mg. per 100 ml., yet when his urine was positive on the day of arrival his blood level had been only 75 mg. per 100 ml.

Exton-Rose dextrose tolerance studies were performed with all but 1 man on the morning following the urine analysis. In 5 the venous blood sugar level rose to 200 mg. per 100 ml. or higher in either the first or the second ½ hour after ingestion of the dextrose. The tolerance tests were then repeated after feeding the subjects liberal amounts of carbohydrate for several days. On repetition not one of the readings in any subject rose above 180 mg. per 100 ml., which is the critical value accepted commonly as top normal[55] for subjects not on carbohydrate-poor intakes. Exton-Rose tests on a series of non-dextrosuric controls yielded essentially identical results. These results with the dextrose tolerance tests are identical with those described earlier in this report, and suggests that a state of temporarily impaired glucose metabolism existed in many of the men at the time of their arrival.

Interviews brought out that the lives of most of the men had been out of balance in the final few days of civilian life. With regularity one learned of hasty meals, farewell parties, gross lack of sleep, alcoholic drinks, family strains, long trips by train or bus, and anticipatory anxiety and excitement. Yet further query brought out that all these emotion stimulating situations held to as great a degree for trainees with sugar-free urines as for those who were voiding sugar.

TABLE 4.—RELATIONSHIPS BETWEEN DIAGNOSIS AND CONCENTRATION OF SUGAR IN INITIAL URINE SPECIMENS

	No. specimens	0.5% or less	0.6% to 1%	1.1% to 2%	Above 2%
Transitory	225	113 (50%)	70 (31%)	36 (16%)	6 (3%)
Low renal threshold	19	7 (36%)	6 (32%)	6 (32%)	0
Renal glycosuria	7	2 (29%)	3 (43%)	1 (14%)	1 (14%)
Diabetes mellitus	21	3 (14%)	5 (24%)	4 (19%)	9 (43%)
Undiagnosed	52	21 (40%)	15 (29%)	13 (25%)	3 (6%)
Totals	324	146 (45%)	99 (31%)	60 (18%)	19 (6%)

Concentration of Sugar in the Urine. The possible relationships between the amount of sugar in the initial urine and the diagnoses ultimately arrived at were evaluated (Table 4). Such wide scatter of the individual readings was found that knowledge of the quantity of sugar in any single specimen of urine would appear of little service as a diagnostic aid. One notes, however, that about half of the transitory cases had sugar concentrations of 0.5% or less, which is the "trace" zone of readings, whereas only a minority of the low renal threshold, renal glycosuria and diabetes mellitus patients exhibited

such weak concentrations. Conversely, of the diabetes patients nearly half had more than 2% sugar in their initial specimens, as opposed to a much lower incidence for the other groups.

Summary. 1. A survey was made of the nature and incidence of melituria as encountered in 77,293 consecutive healthy males at the time of their arrival at a Maritime Service Training Station. Of these, 225 (0.29%) were found to have transitory benign melituria; 19 (0.02%), low renal threshold; 7 (0.01%), persistent renal glycosuria; 52 (0.07%) were undiagnosed; and 21 (0.03%) had diabetes mellitus which had escaped detection in the enrolling offices. No instances of congenital non-dextrosic melituria were recognized.

2. The transitory form of melituria tended to become more frequent with advance in age.

3. The mechanism which provoked the transitory melituria in some subjects on their 1st day of Maritime Service training were far from clear, but emotional influences, fatigue, inflexibility of metabolic adjustments, and poor "carbohydrate saturation" must have played rôles of inconstant and differing importance. The blood sugar was not elevated in many of the subjects at the hour when their urines were positive for sugar.

4. The concentration of the sugar in the urine proved of little aid as a guide to diagnosis.

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THE REACTION OF THE VESSELS OF THE MESENTERY AND INTESTINE TO ANGIOTONIN AND RENIN*†

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It is well known that one of the factors responsible for hypertension is increase in peripheral resistance to blood flow. Recently we have shown[1] that in hypertension of renal origin, the arteries of the rabbit's ear constrict and remain persistently constricted, indicating an increase in peripheral resistance in the somatic area. A similar constriction occurs in acute hypertension produced by injections of angiotonin and renin.[2] The present experiments were performed to find out by direct observation whether or not such constriction occurs in the splanchnic area following injections of these substances, and if so, whether it is of the same degree as that which has previously been shown to occur in the somatic area.

Methods. Four rabbits were used, and in each a portion of the intestine, usually jejunum or ileum, and its attached mesentery was exteriorized in a transparent intestinal mesenteric chamber,[10] using sterile technique. During insertion of the chambers the rabbits were anesthetized with pentobarbital (30 mg./kg. of body weight) injected subcutaneously. The chambers were protected by special shields when not being studied, and remained permanently attached to the rabbit. Intravenous injections of angiotonin and of renin were made 1 to 14 days after insertion of the chambers, and the effects upon the vessels of the mesentery and intestine studied by direct microscopic observation. The arteries and veins were studied at magnifications of from 10 to 30 diameters. The capillaries were studied at magnifications of 200 and 400 diameters, and only those in the mesentery were observed. Ocular micrometric measurements of the vessels were made during control periods of 15 to 30 minutes before injection and the fields were photographed. Following the control observations, angiotonin and renin were injected and the same vessels were again measured and photographed. All injections were made into lateral ear veins.

The arteries of the mesentery studied had control diameters of from 0.12 to 0.42mm., and those of the intestine 0.09 mm. The veins of the mesentery had control diameters of from 0.3 to 0.9 mm. Those of the intestine were not studied.

The renin used was hog renin and was prepared by the method of Helmer and Page.[5] The angiotonin was prepared by the method of Page and Helmer.[8]

Observations. Control. With the exception of rhythmic and spontaneous constriction of the precapillary sphincters and metarterioles, such as described by Chambers and Zweifach,[4] no appreciable changes were observed in the diameters of any of the vessels.

Experimental. 1. *Reaction of the Vessels to Angiotonin.* Fifteen injections of angiotonin were made in amounts varying from 0.2 to 0.5 cc., and the effects upon the arteries, veins and capillaries studied.

Arteries: Fourteen arteries of the mesentery and 2 arteries of the intestine were observed. The arteries all responded similarly. Between 1 and 2 minutes following

* This work was aided by a grant made to the department of anatomy at the University of Pennsylvania by Eli Lilly & Company.

† Brief reference to some of the results given in this paper was made in a report presented before the Physiological Society of Philadelphia, session of May 19, 1942 (Am. J. Med. Sci., **203**, 914, 1942) and in an abstract prepared for the American Association of Anatomists (Anat. Rec., **85**, 330, 1943).

the injections, they constricted 25 to 50% of their control diameters, and returned to their control diameters within 3 to 10 minutes; usually within 3 to 6 minutes after the smaller injections and 5 to 10 after the larger ones.

Veins: The degree of constriction in the veins was not as great as in the arteries. It varied from 10 to 30% of the control diameters. The time of onset of such constrictions was usually between 1 and 4 minutes after the injections. Relaxation did not occur until some minutes later, but the exact time of return of the veins to their normal calibers was not determined.

Typical constriction of the mesenteric arteries and veins caused by injection of angiotonin is shown in Plate I.

Capillaries: The capillaries did not respond visibly. They did not constrict, their lumens remaining open at all times. No appreciable changes were seen in rate of flow through the capillary bed following injections of 0.2 and 0.3 cc. of angiotonin. The injection of 0.5 cc. may have produced a slight reduction in rate of flow, but in any case the blood flow was not interrupted at any time.

2. *Reaction of the Vessels to Renin.* Thirteen injections of renin were made in amounts varying from 0.1 to 0.4 cc., and the effects upon the arteries, veins and capillaries studied.

Arteries: Thirty-two arteries of the mesentery and 3 arteries of the intestine were observed. The arteries all responded similarly; they constricted 40 to 70% of their control diameters after 1 to 2 minutes, and returned to their control diameters after 15 minutes. Thus the constrictions caused by injections of renin lasted in some cases longer than those caused by angiotonin.

In 3 experiments the vessels of the mesentery and intestine were watched with a binocular dissecting microscope at a magnification low enough to permit seeing all of them at once. Following injections of 0.2 cc. of renin all of the arteries were seen to constrict at about the same time.

Veins: The veins also constricted following injections of renin. The degree of constriction varied from 12 to 40% of the control diameters.

Capillaries: The capillaries did not respond visibly. No appreciable changes in rate of blood flow through the capillary bed were observed following injections of 0.1 to 0.2 cc. of renin. Whether or not some reduction in rate of flow followed injection of larger amounts we are not certain. It was possible to determine by direct observation, however, that the blood flow was not interrupted at any time.

3. *Effect of Angiotonin and Renin Upon the Motility of the Intestine.* Injections of both angiotonin and of renin were followed by vigorous peristalsis of the intestine. Such peristalsis usually began during the 2nd minute after the injection and lasted for about 2 to 3 minutes. This peristalsis caused swaying movements of the mesentery, and in consequence some change in arrangement and position of the vessels in the chambers (see Plate I).

In some instances peristalsis was observed during the control periods, and in these cases it was seen that although the vessels of the mesentery and intestine were moved about by it, they did not constrict. Consequently the constriction of the arteries and veins observed in the present experiments was evidently due to the effect of the angiotonin and renin upon the vessels themselves, and not to the movements of the intestine and mesentery.

Discussion. It is obvious from the data given above that the arteries and veins of the mesentery and the arteries of the intestine of unanesthetized rabbits constrict in response to angiotonin and renin when these substances are injected intravenously. The degree and duration of arterial constriction following the injections of angiotonin and renin were approximately the same as in transparent moat chambers in rabbits' ears[2] following injections of the same amounts of these

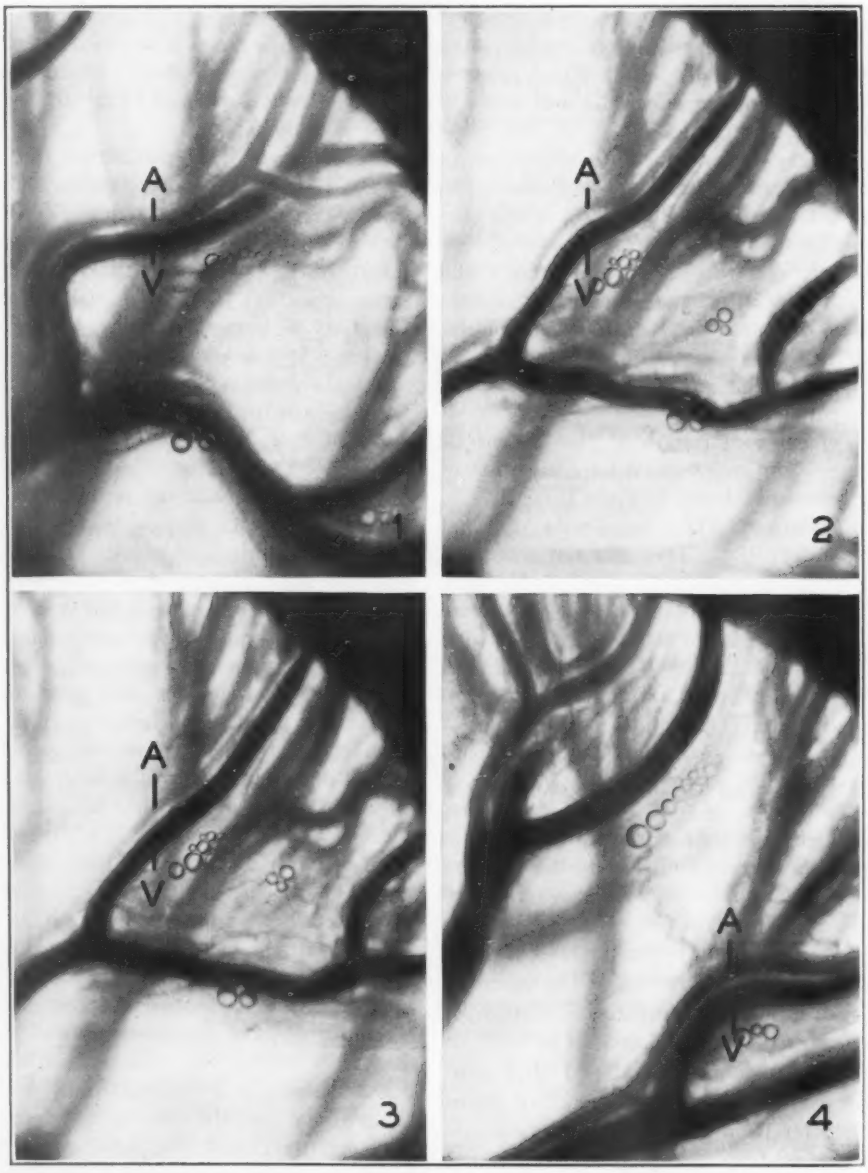

PLATE I.—Photographs of arteries and veins of the mesentery of an unanesthetized rabbit, showing the appearance of the vessels in an intestinal mesenteric chamber before and after intravenous injection of 0.5 cc. of angiotonin. The same artery is indicated by "A" and the same vein by "V" in all of the photographs. The change in positions of the vessels was caused by movements of the mesentery due to the violent peristalsis of the intestine which followed the injection. (× 5.0.) *1*, Before injection. *2*, The same vessels 1½ minutes after the injection. The arteries and veins are constricted. *3*, The same vessels 2½ minutes after the injection. This was the time of maximal arterial constriction. *4*, The same vessels 12 minutes after the injection. The arteries and veins have relaxed to approximately their control diameters.

substances. That the blood flow remained approximately normal in the mesenteric and intestinal vessels in the present experiments following injections of 0.1 to 0.2 cc. of renin and of 0.2 to 0.3 cc. of angiotonin was shown by direct microscopic observations of the flow through the capillary bed, and by absence of blanching of the mesentery and intestine.

The above observations are in accord with the suggestion made by Landis,[6] based upon studies by Landis, Montgomery and Sparkman,[7] that "in contrast to other pressor substances the active material in kidney extracts increases the tone of all the arterioles throughout the body more or less equally. Since in each vascular bed peripheral resistance and arterial pressure rise proportionally, blood flow remains approximately normal."

Constriction of the veins in the present experiments was anticipated because it had been observed in the rabbit's ear following injections of renin and angiotonin,[2] and because veins of the size studied contain appreciable amounts of muscle. Furthermore, it had been shown that angiotonin and renin cause constriction of arterioles lacking a nerve supply, and hence presumably angiotonin has a direct action upon the vascular muscle. It is of interest in this connection that Wilkins and Duncan[9] observed a rise in venous pressure in normal persons in which hypertension had been induced by injection of angiotonin.

The present studies demonstrate constriction of arteries in the living animal following injection of renin and angiotonin. Previous studies by Bingle and Strauss[3] show that the isolated beef artery likewise constricts upon addition of active kidney extracts.

It has been reported previously that the capillaries in transparent chambers in rabbits' ears do not constrict following injections of angiotonin and renin,[2] and the present studies show that in the mesentery they likewise fail to constrict.

Since the arteries of the mesentery and intestine constrict following injections of angiotonin and renin, it seems not unlikely that they constrict also during chronic hypertension of renal origin. As stated previously, during the development of hypertension of renal origin the arteries in the rabbit's ear constrict, and remain persistently constricted during the period of hypertension, thus increasing the resistance to blood flow. The present work suggests that during such hypertension, arterial constriction in the splanchnic area may also play a part in increasing the resistance to blood flow.

Summary. 1. The present experiments demonstrate by direct microscopic observation that both angiotonin and renin, when injected intravenously, cause constriction of the arteries of the mesentery and intestine of unanesthetized rabbits. The veins of the mesentery also constricted. The veins of the intestine were not studied. Neither substance caused constriction of the capillaries, nor any appreciable change in rate of blood flow, as shown by direct observation of the capillary bed; nor did either substance produce blanching of the mesentery and intestine.

2. Both angiotonin and renin, injected intravenously, caused vigorous peristalsis of the intestine.

We are indebted to Mr. B. B. Varian for the photographs.

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SULFAPYRAZINE—ITS USE IN THE PROPHYLAXIS OF RESPIRATORY DISEASE

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Conditions peculiar to military operations frequently have operated to produce excessively high rates of respiratory disease in general and of streptococcal disease in particular. In many instances the loss of man-days caused by this type of disease had become such a serious barrier to the effective prosecution of the war effort that it became expedient to attempt to devise procedures that might effectively lower these disease rates. For this reason, and based on assumptions implicit in the literature,[3,4,5] the prophylactic use of small daily dosages of sulfonamides for the control of respiratory diseases has been tried and has met with considerable success in the desired temporary goal.

Of the sulfonamides that have been chosen for this purpose, sulfadiazine has been the drug of almost universal choice. Numerous experiences have attested to the fact that its use has been attended not only with success in lowering the respiratory disease rate but also with a surprisingly low incidence of serious toxic reactions.[3,4,5] Unfortunately, however, during the winter of 1944–1945, strains of Group A, Type 17 streptococcus highly resistant to sulfadiazine were recognized with increasing frequency and were responsible for relatively alarming outbreaks of streptococcal diseases. As might be expected, the use of sulfadiazine for either prophylactic or therapeutic purposes proved ineffective in controlling diseases due to this group of organisms.

The mechanism of acquired resistance by an organism to a chemotherapeutic agent is as yet unclarified. However, contrary to previous ideas gained largely through a study of "sulfonamide fastness" induced *in vitro*, preliminary observations[8] have indicated that organisms naturally resistant to 1 type of sulfonamide may retain their sensitivity to other sulfonamide drugs. If these observations prove to be true, it might be possible effectively to employ 1 sulfonamide for prophylaxis against organisms resistant to the prophylactic activity of another sulfonamide drug. Or it appears conceivable that, if prophylaxis with one type of sulfonamide is accompanied by hospital admissions due to disease caused by organisms resistant to this particular drug, another type of sulfonamide might be used for the treatment of such patients.

Moreover, whereas sulfadiazine prophylaxis has been repeatedly demonstrated to be both effective (in the absence of sulfadiazine-resistant strains) and relatively non-toxic, it is possible that other sulfonamide drugs might be used with even greater effectiveness and with less dangerous side reactions. Accordingly, it appeared important to evaluate the results obtained with the use of other sulfonamides for the chemoprophylaxis of respiratory infections.

Preliminary clinical studies have indicated that sulfapyrazine[1,6,7] was effective in the treatment of pneumococcal disease and that its use was attended by a very low rate of toxic reactions. Whereas a relatively small number of patients had been treated successfully with therapeutic dosages over short periods of time, to our knowledge this drug has never been used for chemoprophylaxis and no data were available concerning its effects on man

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when given in daily small dosages over an extended period of time. It therefore appeared desirable to acquire data concerning sulfapyrazine prophylaxis. Accordingly, the effectiveness of sulfapyrazine for the chemoprophylaxis of respiratory disease was investigated.

Methods. This study was performed at an Army Air Base during the winter months of 1944–45. At this Field, large groups of soldiers received a 26 week course of instruction and it was therefore possible to study respiratory disease rates in a comparatively stable population over a significantly long period of time. Moreover, due to its location and climatic conditions, this Field had experienced, in the past, relatively high respiratory disease rates and it was also known, from previous studies, that a Group A, Type 17 hemolytic streptococcus had long been endemic.

From the population of this Field, 4 separate squadrons, each containing approximately 1000 men, were chosen to receive prophylaxis. Two of these squadrons, Squadrons H and L were located in one area of the Field. The other 2, Squadrons N and O were both located in a widely separated region of the same Field. All 4 squadrons were quartered in similar cantonment type wooden barracks. The personnel of Squadrons H and L used the same mess, classrooms, theater, etc., so that a necessary amount of intermingling occurred. The same was true for the personnel of Squadrons N and O. However, the administrative routine of the Field was such that no intermingling occurred between the personnel of Squadrons H and L, on the one hand, and the personnel of Squadrons N and O, on the other hand. For the purposes of this investigation, only student personnel of these 4 squadrons were studied as the general environmental and living conditions of the attached permanent party personnel were not comparable.

Since this study was designed to compare the relative effectiveness of sulfapyrazine with sulfadiazine and since, for the reasons enumerated above, Squadron L was regarded as comparable to Squadron H and Squadron N comparable to Squadron O, Squadrons L and O were chosen to receive prophylaxis with sulfapyrazine whereas Squadrons H and N received sulfadiazine. It was believed that it would thus be most feasible to compare the relative activities of the 2 different drugs.

The respiratory disease rates were determined for all 4 squadrons during a 4 week control period. At the end of that time, Squadron H was given sulfadiazine and Squadron L sulfapyrazine prophylaxis for a 30 day period. Ten days later, Squadron N was started on sulfadiazine and Squadron O on sulfapyrazine prophylaxis for the following 30 days. The personnel of all 4 squadrons each received 1 gm. per day of the appropriate drug.

The administration of the drug was accomplished with the excellent coöperation of the Squadron Commanders, who made it a part of the Barracks Chiefs' duties to administer the daily dosage and to certify that it was actually swallowed while in their presence. A negligible number of men was excused from taking the drugs due to a previous history of sulfonamide sensitivity. Numerous unannounced spot checks were made using the simple so-called "newspaper test"[2] which was capable of detecting even very minute quantities of sulfonamides in the urine. Blood specimens drawn at intervals from representative samples of the squadrons showed traces only of sulfapyrazine, but since our only object was to determine whether or not the drug was being taken, we were satisfied with the results of the urine test. On only 1 or 2 occasions as indicated by this test was anyone found who had not been taking the drug.

A number of pertinent facts were recorded and analyzed: 1. Complete records were kept of all hospital admissions for acute respiratory disease.*

2. In order to ascertain the influence of prophylaxis on streptococcal disease in particular, throat cultures were routinely taken on all hospital admissions within a maximum

* In this study acute respiratory disease was used to include both upper and lower respiratory infections, including nasopharyngitis (both bacterial and non-bacterial), laryngitis, tonsillitis, scarlet fever, bronchitis, pneumonia (bacterial and non-bacterial) influenza and other virus infections of the respiratory tract. It did not include any chronic infections such as chronic bronchitis, tuberculosis, chronic sinusitis, etc. In addition re-admission for complications of acute respiratory disease such as mastoiditis, otitis media, etc., were not considered as separate admissions.

of 15 hours after their entrance to the hospital wards and prior to any treatment.

3. Hospital admission rates reflected the trends of relatively severe and disabling respiratory disease. On the other hand, numerous personnel suffered from mild infections not considered severe enough for their hospitalization. It was felt accordingly that a somewhat more revealing study of the trend of respiratory disease rates could be accomplished by recording all dispensary admissions seeking aid for respiratory disease. Accordingly a tabulation was kept on all dispensary admission rates for respiratory disease. In this study, therefore, the dispensary admissions were used as an index of *total* respiratory diseases.

4. In order to determine the effect of prophylaxis on carrier rates among the healthy personnel, weekly throat cultures were taken throughout the control period and for the duration of the prophylaxis in the squadrons included in this study.

5. All personnel receiving prophylaxis was carefully watched for any evidences of drug reactions. All men had been instructed to report immediately to the dispensaries at the first signs of any of the symptoms known to occur with the use of sulfonamides. Those individuals who developed any possible symptoms of reaction were removed temporarily, at least, from further prophylaxis and were referred to the dermatologist for appropriate study. The latter recorded the type of reaction, its severity and duration and determined the ability of the patient to react to further small doses of the same or different sulfonamide.*

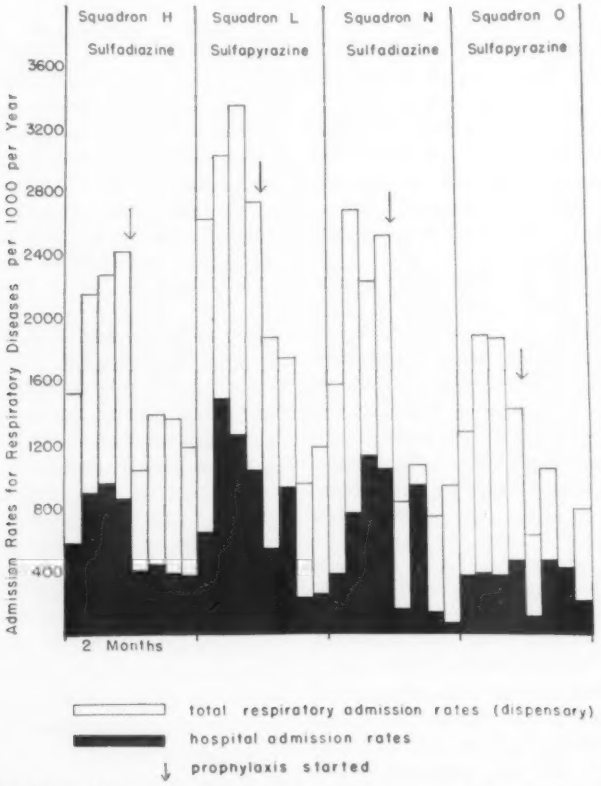

FIG. 1.—Effects of sulfapyrazine and sulfadiazine prophylaxis on respiratory disease rates by weeks.

* We are indebted to Major Ben A. Newman, M.C., for his invaluable aid in these particular studies.

Results. A. *Effect of Respiratory Disease Rates.* The effects of the prophylaxis on respiratory diseases were evidenced by a comparison of the admission rates for both hospital and dispensaries during the control period preceding prophylaxis and for the 30 day period of prophylaxis. These figures revealed that the institution of both sulfapyrazine and sulfadiazine resulted in a marked and abrupt decline in the rates for all acute respiratory diseases. A graphic comparison of these rates is given in Figures 1 and 2.

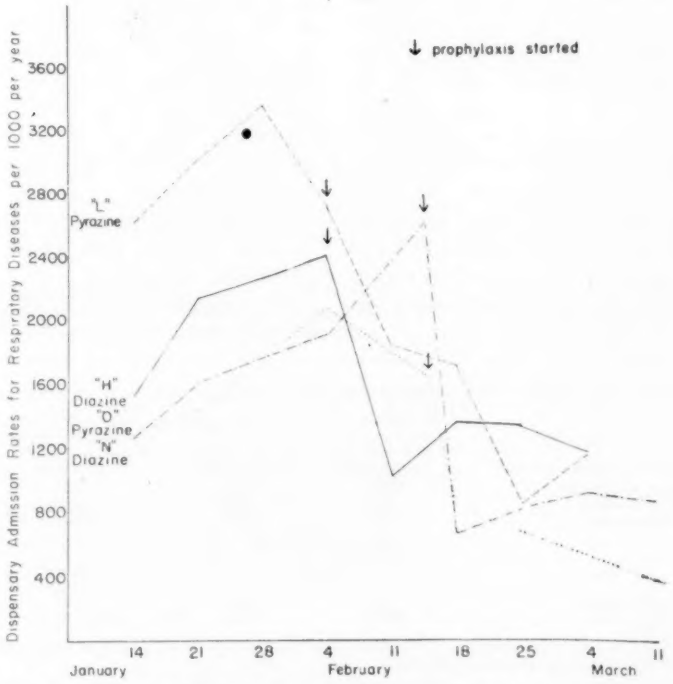

FIG. 2.—Effects of prophylaxis on respiratory disease rates in four squadrons.

Figure 1 demonstrates a marked and uniform drop in hospital admission rates in Squadron H during the period of sulfadiazine prophylaxis. In Squadron L (sulfapyrazine) and Squadron N (sulfadiazine) the decreases in hospital admission rates were also marked although, in each instance, during the 2nd week of prophylaxis, there was a temporary rise in these rates. In Squadron O, which received sulfapyrazine, the hospital admission rates were comparatively low both before and during prophylaxis and the differences resulting from prophylaxis did not appear significant.

However, as noted above, hospital admissions reflected only moderately severe and severe cases. A somewhat more accurate picture of the effects of prophylaxis was obtained through comparison of the dispensary admission rates for respiratory diseases inasmuch as these latter figures more closely revealed the total number of respiratory infections. These figures for dispensary rates are also given in Figures 1 and 2 and demonstrate that all 4 squadrons witnessed a marked decline in respiratory disease coincident with the inception of both sulfapyrazine and sulfadiazine prophylaxis.

Numerous facts tend to substantiate the conclusion that the decline in respiratory disease coincident with prophylaxis could be attributed solely to the effects of the latter and not to the spontaneous disap-

pearance of an epidemic. In the first place, studies from previous years had established the fact that the epidemic period of respiratory disease at the Field did not end spontaneously until May, whereas the decline witnessed during this particular investigation occurred in a season (February and March) during which the rates could be predicted to increase or remain at their already high levels. In the second place, prophylaxis was instituted in 2 of the squadrons 10 days after its inception in the other 2. Figure 2 demonstrates that the rates in the 2 squadrons not yet receiving prophylaxis continued to remain at high levels during this interval, whereas the rates for the other 2 squadrons had already declined. However, immediately after the institution of prophylaxis in the last 2 squadrons, the respiratory disease rates for these groups experienced the same sharp decrease. Again, while not conclusive, these facts bring additional weight to the argument that the declines in respiratory diseases were not spontaneous.

A third point of evidence consisted in a comparison between the admission rates for respiratory diseases in the experimental squadrons and in other squadrons not chosen to receive the benefit of prophylaxis. Those figures are found in Table 1 wherein the hospital admission rates for respiratory disease in the 2 squadrons receiving sulfadiazine prophylaxis and in the 2 squadrons receiving sulfapyrazine prophylaxis are compared with the rates, first for 4 other squadrons and, secondly, for the entire remainder of the post.

It was not to be expected that these latter rates would be strictly comparable to those of the squadrons receiving prophylaxis. Due to the plan for operation of the Field, no squadrons other than the ones chosen to receive prophylaxis could be selected to form a comparable epidemiologic unit.* Due to different duties, different housing conditions, different durations of stay on the Field, etc., conditions in the 4 untreated squadrons were such as to result in respiratory disease rates somewhat lower than those found in the squadrons receiving prophylaxis. Furthermore, the total of those units comprising the entire remainder of the post consisted largely of well-seasoned personnel with duties and housing conditions entirely different from those of the 4 experimental squadrons.

It is recognized that epidemics originate and are initially confined to a single barrack and then spread through an entire squadron. Due to the quantitative differences in exposures of members of other squadrons from those of the affected squadron, rates for respiratory diseases might be expected to vary. These quantitative differences were such as to maintain respiratory disease rates among the 4 untreated squadrons at somewhat lower,

TABLE 1.—COMPARISON OF SULFADIAZINE AND SULFAPYRAZINE PROPHYLAXIS

Respiratory disease rates*	Squadrons receiving sulfadiazine		Squadrons receiving sulfapyrazine	
	4 wks. preceding prophylaxis	4 wks. during prophylaxis	4 wks. preceding prophylaxis	4 wks. during prophylaxis
Dispensary admissions	2139	1093	2176	1048
Hospital admissions	807	364	685	391
Average population	2030	2070	1840	1860
	Untreated Controls 4 Squadrons†		Remainder of Post†	
Hospital admissions	459	439	282	312
Average population	4360	4145	Restricted	Restricted

* Rates expressed in terms of admissions per 1000 per year for the 4 week period under consideration.
† Figures not available for dispensary admissions.

* The ideal method to establish control units for those receiving prophylaxis would have been to divide each squadron into 2 halves and to have reserved one of these halves for prophylaxis while the other half received none. Unfortunately, inter-barracks shifts of personnel within the same squadron were so necessary that, according to the Squadron Commanders, such controls could not be maintained.

and for the entire remainder of the post, at markedly lower figures than those found in the squadrons chosen to receive prophylaxis. Nevertheless, a necessary amount of intermingling occurred between personnel of the squadrons receiving prophylaxis and the personnel of the remainder of the post. Accordingly, any natural trends affecting the respiratory disease rates in 1 group of squadrons would be expected to be reflected sooner or later in the other groups provided the comparison was made over a significantly long period. The figures in Table 1 indicate, however, that whereas a markedly significant decrease in respiratory disease rates occurred in the experimental squadrons during the period of prophylaxis, rates for the remainder of the Field were such as to warrant the conclusion that the marked decreases in respiratory disease rates observed in Squadrons H, L, N and O were not due to any spontaneous, cyclic variations.

strated that not only streptococcal diseases but also that other respiratory diseases, even those thought to be caused by agents not susceptible to sulfonamides, were decreased as a result of prophylaxis. The explanation for this fact is not clear. However, in this particular study, the same findings were observed. The use of sulfonamides was expected to produce its greatest effect on those diseases caused by organisms sensitive to these drugs. Such diseases include all those caused by the beta-hemolytic streptococcus and the gonococcus.* As a result of the routine throat culture studies on all hospital admissions, it was possible to differentiate the respiratory diseases of non-streptococcal origin from those caused by the streptococcus.

In Table 2 a comparison is made of the effects of both sulfapyrazine and sulfadiazine prophylaxis on the admissions due to organisms definitely susceptible to sul-

TABLE 2.—COMPARISON OF SULFAPYRAZINE AND SULFADIAZINE PROPHYLAXIS ON DISEASES CAUSED BY STREPTOCOCCI AND GONOCOCCI AND DISEASES NOT REGARDED SUSCEPTIBLE TO SULFONAMIDE THERAPY

Types of disease	Sulfadiazine		Sulfapyrazine	
	4 wks. preceding prophylaxis*	4 wks. during prophylaxis*	4 wks. preceding prophylaxis*	4 wks. during prophylaxis*
Total cases caused by streptococcus and gonococcus	29	10	34	11
Strep. pharyngitis	13	5	10	5
Scarlet fever	11	2	16	2
Gonorrhea	5	3	8	4
Non-streptococcal respiratory disease	102	51	71	49

Untreated Control—Remainder of Post

	4 wks. preceding prophylaxis*	4 wks. during prophylaxis*
Scarlet fever	39	80
Gonorrhea	57	59

* Figures represent actual number of cases.

Accordingly, by virtue of the various arguments advanced above, it is believed that the weight of the evidence leads to the conclusion that the decline in respiratory disease rates in the squadrons receiving prophylaxis could be attributed quantitatively to the use of chemoprophylaxis.

In previous experiences with sulfonamide prophylaxis[3,4,5] it has been demonfonamides and the admissions resulting from organisms and viruses not susceptible to the level of chemotherapy used in this study. It will be observed that sulfapyrazine and sulfadiazine were both markedly effective in reducing the number of admissions due to scarlet fever, other streptococcal respiratory diseases and gonorrhea. It is also apparent, however, that the incidence of respiratory diseases other

* No cases of meningitis were observed during the entire period of study. Sulfonamide prophylaxis in the dosages used here, did not appear effective against pneumococcal pneumonia.

than those enumerated above also was decreased coincident with the use of both types of prophylaxis. Table 2 also demonstrates that the incidence of streptococcal disease and of gonorrhea underwent no spontaneous decline in the remainder of the Field.

TABLE 3.—TABLE OF DIFFERENCES FOR 4 WEEKS PRIOR TO AND 4 WEEKS DURING PROPHYLAXIS

	2 diazine squadrons	Squadron O pyrazine	Squadron L pyrazine	4 untreated control squadrons
Total hospital respiratory disease admissions	68	9	32	14
Total hospital strep. respiratory disease admissions	17	2	17	7
Total gonorrhea cases	2	1	3	*
Total respiratory disease dispensary admissions	160	69	89	*
Average population	2050	1018	833	4253

* Figures not available.

A statistical analysis of the various results enumerated above was kindly performed by Lt. Arthur Kemp, A.C., of the Department of Statistics, AAF School of Aviation Medicine. Table 3 gives the differences between 4 weeks prior to and 4 weeks during prophylaxis for total (dispensary) respiratory diseases, hospital admissions for respiratory diseases and hospital admissions for streptococcal disease plus gonorrhea. It was found that both squadrons receiving sulfadiazine prophylaxis were comparable and could be used as a single unit for statistical comparison. However, for reasons enumerated above, the 2 squadrons which received sulfapyrazine prophylaxis were not found to be comparable. Accordingly, it became necessary to compare each of the squadrons receiving sulfapyrazine with the other groups.

The method used consisted of an analysis of the significance of the differences between the proportions of admissions of the several kinds to their respective populations.

These figures reveal that when the rates for Squadron L (sulfapyrazine) were compared with those for both squadrons receiving sulfadiazine, sulfapyrazine was significantly more effective than sulfadiazine in producing a decrease in dispensary admission rates ($t = 2.82$) and a decrease in diseases caused by the streptococcus and gonococcus ($t = 2.71$). On the other hand, there was no significant difference between sulfapyrazine in Squadron L and sulfadiazine in regard to reduction of hospital admission rates for all acute respiratory diseases ($t = 1.05$).*

However, a comparison of the rates for the other squadron receiving sulfapyrazine (Squadron O) with those for the 2 squadrons receiving sulfadiazine shows no significant differences between sulfapyrazine and sulfadiazine in regard to reduction of dispensary admission rates for respiratory disease ($t = 1.20$) and for diseases caused by the streptococcus and gonococcus ($t = 1.9$). On the other hand, as expected, sulfadiazine was significantly more effective than sulfapyrazine in Squadron O in regard to reduction of hospital admission rates for acute respiratory diseases ($t = 4.18$).

These analyses give somewhat conflicting results between the 2 squadrons receiving sulfapyrazine and those partaking of sulfadiazine prophylaxis. It therefore must be concluded that the results of this study failed to produce evidence of the relative superiority of either sulfadiazine or sulfapyrazine for the prophylaxis of respiratory diseases. It is obvious that future studies involving much larger groups are required for the solution of this problem.

Those cases of streptococcal disease which were admitted to the hospital from the squadrons receiving both sulfapyrazine and sulfadiazine prophylaxis proved principally to be due to a Group A, Type 17 streptococcus. Sensitivity stud-

* t = the critical ratio of the difference between 2 proportions divided by the standard error of the difference. In order to be significant at the 1% level, *i. e.*, in order to have less than 1 chance in 100 that a difference as large as that observed is due to chance, the value of t must exceed 2.58.

ies were performed on these organisms with somewhat conflicting results inasmuch as a number of them proved to be sensitive to both sulfonamides whereas others were relatively resistant. No detailed analysis is appended but sulfapyrazine and sulfadiazine appeared to be about equally ineffective in controlling disease caused by this particular organism.

An analysis of the carrier rates is beyond the scope of this paper because of the presumed relationship between carrier rates and resistance to the drug. For this reason it will be sufficient to mention only that the carrier rates in the squadrons receiving prophylaxis did not recede sharply following its introduction.

Table 4.—Toxic Reactions to Sulfonamide Prophylaxis

Drug used	Type of reaction	Appearance in days after start of prophylaxis	Duration of symptoms	Previous reactions to sulfonamides	Remarks
Sulfapyrazine	Cutaneous—urticarial	1	3	None	Previous urticaria to A.P.C. capsules
Sulfapyrazine	Cutaneous—morbilliform	4	10	Yes	Fever and chills with diazine prophylaxis 1 year previous
Sulfapyrazine	Cutaneous—fixed drug reaction	7	5	Yes	Similar previous reaction to diazine 1 year previous
Sulfadiazine	Cutaneous—fixed drug reaction	1	7	None	Readministration of 1 gm. diazine reproduced reaction; 1 month later tolerated 7 daily gm. doses of sulfapyrazine
Sulfadiazine	Cutaneous—erythema multiforme	16	5	None	Readministration of 1 gm. daily of pyrazine for 3 days reproduced rash
Sulfadiazine	Cutaneous—scarlatiniform	1	13	None	
Sulfadiazine	Cutaneous—urticarial	8	6	None	Readministered pyrazine 1 gm. daily for 6 days with no reaction
Sulfadiazine	Conjunctivitis	1	5	Yes	Previous similar reaction to diazine 1 year ago
Sulfadiazine	Granulopenia, headaches	5	10	None	Recovered spontaneously; not very ill
Sulfadiazine	Nausea, cramps, diarrhea	7	4	None	Readministration of diazine produced same reaction after 6 days; pyrazine 1 gm. per day for 7 days was tolerated without reaction

Of the 7 men reacting to sulfadiazine, 4 showed cutaneous manifestations, 1 had nausea and diarrhea, 1 presented a transient neutropenia accompanied by headache and 1 displayed a conjunctivitis. The case of neutropenia improved spontaneously, the neutrophils increasing from 15 to 60% in 7 days. The individual developing conjunctivitis a few hours after his initial dose of sulfadiazine reported a similar episode following the previous use of sulfadiazine.

B. *Toxic Manifestations.* Approximately 2000 men received 1 gm. daily doses of either sulfapyrazine or sulfadiazine for a total duration of 30 days. The total number of reactions observed in both groups and the types of reactions are given in Table 4. It is apparent that there was a total of 10 reactions, 7 in the sulfadiazine and 3 in the sulfapyrazine treated group. In accord with other experiences with sulfonamide prophylaxis, serious reactions were conspicuous by their absence.

The majority of reactions (Table 4) were cutaneous in type. All of the sulfapyrazine reactions belonged to this category and presented manifestations lasting from 3 to 11 days. In this group there was 1 urticarial reaction, 1 moribilliform eruption and 1 case of erythematous patches in areas of lichenification which had resulted from a former drug reaction. All 3 of these individuals had taken sulfadiazine at some previous period and 2 of these had experienced reactions to this drug at an earlier date. The patient with urticaria, however, failed to recall a previous sulfonamide reaction.

The time for the development of these reactions varied considerably. The case of conjunctivitis mentioned above occurred a few hours after the initial dose and the longest interval between start of

prophylaxis and appearance of the reaction was 16 days.

Three of the individuals reacting to sulfadiazine were given trial doses of sulfapyrazine after the initial symptoms had disappeared. One case, involving a scarlatiniform eruption, had a similar reaction after 3 gm. daily of sulfapyrazine. The other cases (urticaria and nausea) were able to take 6 and 7 daily gm. respectively of sulfapyrazine without reappearance of symptoms.

Summary and Conclusions. 1. Daily doses were administered over a 30 day period to 2 comparable military groups—1 gm. daily of sulfapyrazine to 1 group, 1 gm. of sulfadiazine to the other.

2. Sulfapyrazine and sulfadiazine were both effective in decreasing streptococcal and non-streptococcal respiratory disease rates.

3. Some indication was obtained that sulfapyrazine prophylaxis was slightly less toxic than sulfadiazine prophylaxis.

Grateful appreciation is due Dr. Warren Cox of Mead Johnson & Co. for the generous supply of sulfapyrazine used in this study. Sgt. William Wolf rendered invaluable aid in administrative problems.

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VITAMIN LEVELS IN PERNICIOUS ANEMIA*

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As yet, few quantitative studies concerning the presence or absence of vitamin deficiencies in pernicious anemia have been reported. In the past few years, laboratory procedures have been devised for the measurement of vitamin levels in the blood and urine and are now fairly well standardized. Vitamins A and C and carotene can be determined with reasonable accuracy on blood plasma. The B complex vitamins are measured in the urine by chemical and biologic assay, before and after the administration of a test dose. From previously reported studies,[1] there appears to be a definite relationship between the early clinical manifestations of a B complex deficiency and the vitamin levels as determined in the laboratory. The application of these new procedures to patients having pernicious anemia should afford a more exact method of study of this disease and enable one to determine the presence or absence of vitamin depletion in such patients.

Material. Eight patients having pernicious anemia were studied, 7 of whom had the characteristic hematologic picture, as well as glossitis, papillary atrophy of the tongue, and early neurologic changes. The 8th patient was in remission at the time of this study. Two had a diarrhea of from 10 to 15 stools per day. The oldest patient was 65; the youngest, 37. There was an equal number of males and females. No evidence of other organic or debilitating disease was demonstrable.

For the purpose of comparison, 2 other groups were studied: (1) 25 patients diagnosed clinically as having a B complex deficiency; and (2) 30 medical students who were used as normal controls.

Method. The method of study was similar to that described in a previous communication.[1] All patients were hospitalized and given a standard diet adequate in protein and calories, but low in vitamins. The approximate composition of the diet was: protein, 60 gm.; fat, 100 gm.; carbohydrate, 325 gm.; total calories, 2500. The estimated vitamin content of this diet was: vitamin A, 130 international units; vitamin C, less than 10 mg.; thiamin, 300 gamma; riboflavin, 250 gamma; nicotinic acid, less than 5 mg.

Vitamin C was measured in the Evelyn photoelectric colorimeter using Tillman's dye and the technique of Mindlin and Butler.[2] Vitamin A determinations were made by the method of Kimble.[3] The vitamins of the B complex were determined in the urine before and after the following test doses: thiamin, 1 mg. intramuscularly; riboflavin, 5 mg. orally; nicotinic acid amide, 500 mg. orally; pyridoxine, 50 mg. orally. Thiamin was first determined by the yeast fermentation technique of Atkins, Schultz, and Frey,[4] and later by a modification of the thiochrome method. Riboflavin was measured by direct fluorometric method of Ferrebee,[5] omitting the adsorption and elution steps. The nicotinic acid load test was done as described by Perlzweig, Sarett and Margolis.[6] F_2 values were determined fluorometrically and paralleled those for nicotinic acid excretion. Pyridoxine was determined colorimetrically by the technique of Scudi *et al.*[7]

Results. Vitamin A. None of the group studied had any of the findings usually

* This study was supported by a grant from the John and Mary R. Markle Foundation and the Anna H. Hanes Research Fund.

attributed to a vitamin A deficiency. Although the plasma levels of A in the deficiency group and in the patients having pernicious anemia were below those of the normal controls, still the means of these 2 groups were above the suggested lower limit of normal and were essentially the same (Fig. 1). Although the carotene values in the pernicious anemia patients were lower than those of the

TABLE 1.—ARITHMETICAL MEANS OF VITAMIN LEVELS

Vitamins*	Student controls	B complex deficiencies	Pernicious anemia patients	Suggested lower limit of normal
A	114	89	85	75
Carotene	265	205	154	165
C	0.67	0.33	0.42	0.15
Nicotinic acid	88	48	60	65
Thiamin (control period)	668	454	529	400
Thiamin (load test)	422	152	203	300
Riboflavin (control period)	810	387	399	400
Riboflavin (load test)	3333	2127	2375	2200
Pyridoxine	7	5	6.5	4.7

* Vitamin A—International Units per 100 cc. of plasma.
Carotene—International Units per 100 cc. of plasma.
Vitamin C—Mg. per 100 cc. of plasma.
Nicotinic acid—Urinary excretion in mg. in 14 hours after 500 mg. oral test dose.
Thiamin—Control period—Urinary excretion in gamma in 24 hours.
Load test—Urinary excretion in gamma in 24 hours after 1 mg. intramuscularly.
Riboflavin—Control period—Urinary excretion in gamma in 24 hours.
Load test—Urinary excretion in gamma in 24 hours after 5 mg. orally.
Pyridoxine—Urinary excretion in mg. in 4 hours after 50 mg. orally.

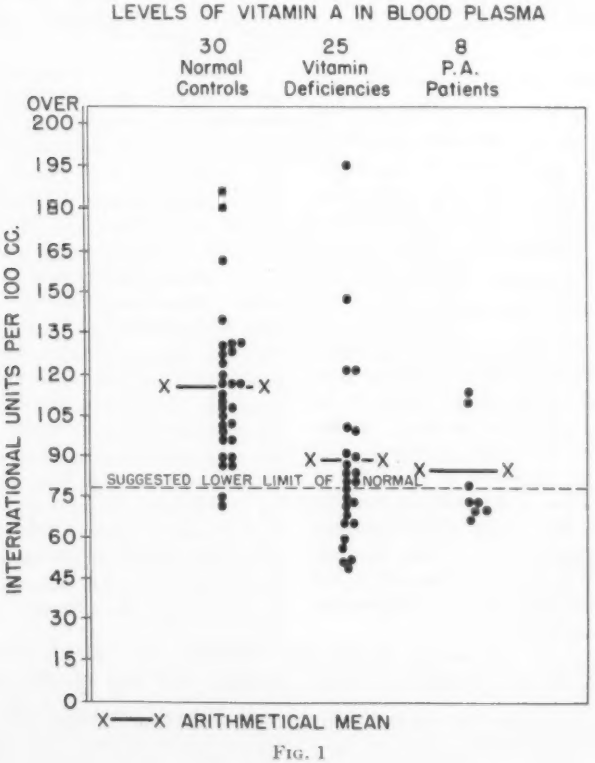

FIG. 1

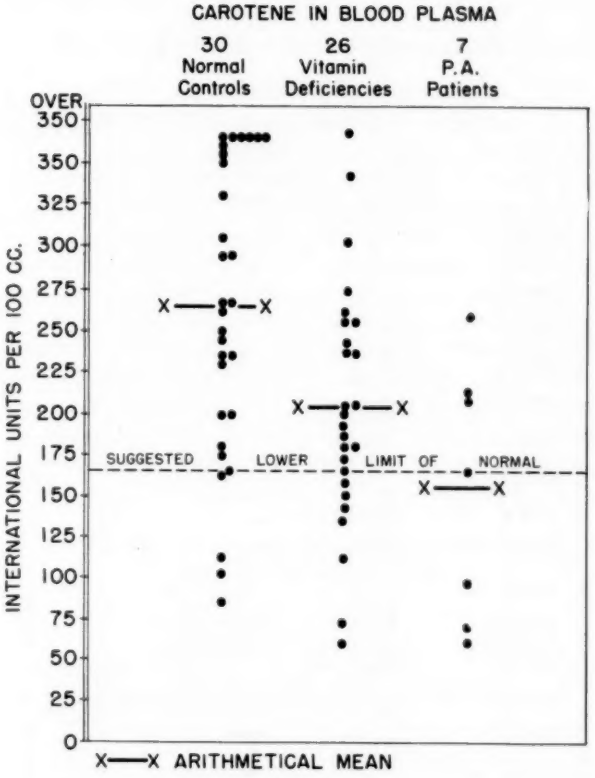
CAROTENE IN BLOOD PLASMA
30
Normal
Controls
26
Vitamin
Deficiencies
7
P.A.
Patients
OVER
350
350
325
300
275
250
225
200
175
150
125
100
75
50
25
0
INTERNATIONAL UNITS PER 100 CC.
SUGGESTED LOWER LIMIT OF NORMAL
X—X ARITHMETICAL MEAN

FIG. 2

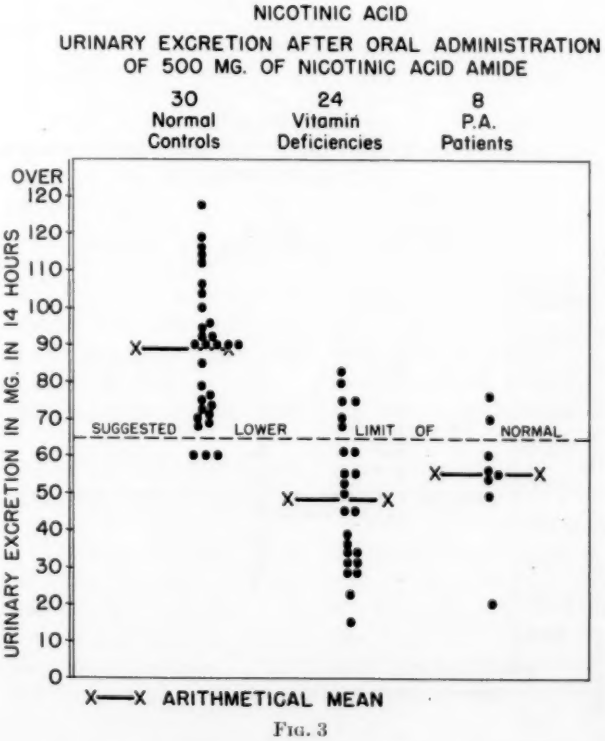
NICOTINIC ACID
URINARY EXCRETION AFTER ORAL ADMINISTRATION
OF 500 MG. OF NICOTINIC ACID AMIDE
30
Normal
Controls
24
Vitamin
Deficiencies
8
P.A.
Patients
OVER
120
120
110
100
90
80
70
60
50
40
30
20
10
0
URINARY EXCRETION IN MG. IN 14 HOURS
SUGGESTED LOWER LIMIT OF NORMAL
X—X ARITHMETICAL MEAN

FIG. 3

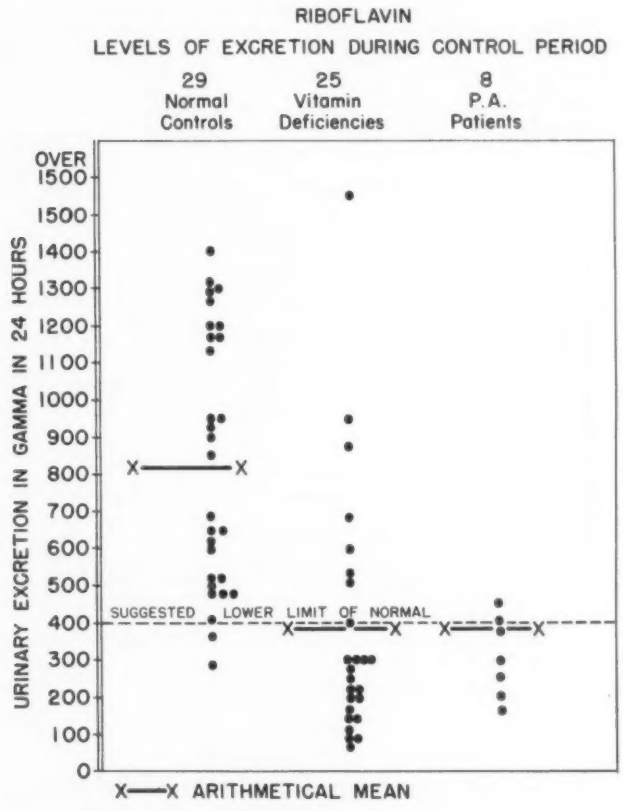
RIBOFLAVIN
LEVELS OF EXCRETION DURING CONTROL PERIOD
29 Normal Controls
25 Vitamin Deficiencies
8 P.A. Patients
URINARY EXCRETION IN GAMMA IN 24 HOURS
OVER 1500
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
SUGGESTED LOWER LIMIT OF NORMAL
X—X ARITHMETICAL MEAN

FIG. 4

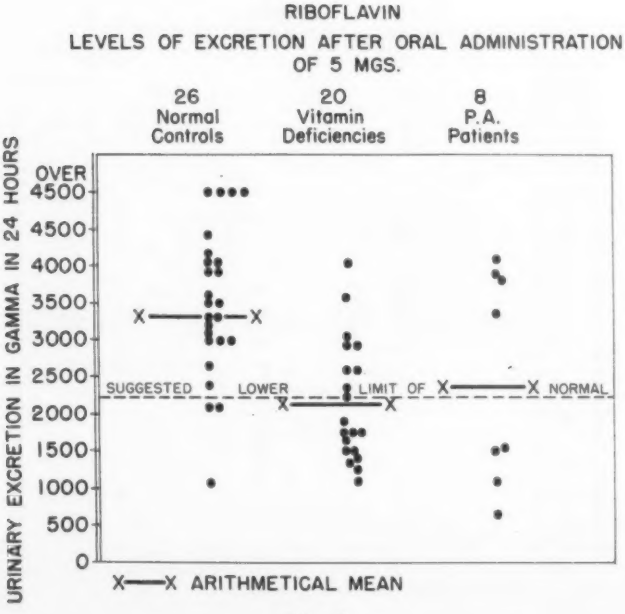
RIBOFLAVIN
LEVELS OF EXCRETION AFTER ORAL ADMINISTRATION OF 5 MGS.
26 Normal Controls
20 Vitamin Deficiencies
8 P.A. Patients
URINARY EXCRETION IN GAMMA IN 24 HOURS
OVER 4500
4500
4000
3500
3000
2500
2000
1500
1000
500
0
SUGGESTED LOWER LIMIT OF NORMAL
X—X ARITHMETICAL MEAN

FIG. 5

deficiency group, still the difference was not statistically significant (Fig. 2).

VITAMIN C. There were individuals in each group whose plasma contained no measurable vitamin C. A comparison of the distribution between groups, however, showed no marked difference.

NICOTINIC ACID. A comparison of the urinary excretion of nicotinic acid in the 3 groups, following the administration of 500 mg., is shown in Figure 3. It will be noted that the mean of the patients having pernicious anemia was essentially the same as that of the deficiency group, but both were considerably lower than that of the normal controls. Two-thirds of the patients with pernicious anemia, as well as those having a B complex deficiency, had values below the suggested lower limit of normal.

RIBOFLAVIN. The distribution of values for riboflavin excretion before and after the test dose is shown in Figures 4 and 5. The pernicious anemia group had values which were practically the same as those of the patients having a B complex deficiency. Both were significantly lower than those of the normal controls.

THIAMIN. Before and after the test dose, the thiamin levels in the pernicious anemia patients were practically the same as those in the deficiency group, the distribution in general following that of riboflavin. Again, both fell well below the normal control values (Fig. 6).

PYRIDOXINE. There were no significant differences in the distribution of values of pyridoxine excretion between normal individuals and those with pernicious anemia.

Discussion. It is generally recognized that the glossitis, cheilosis, papillary atrophy of the tongue, and parasthenia of the extremities which one sees in patients having pernicious anemia and sprue are frequently indistinguishable from those same findings when they occur in patients having a frank B complex deficiency. It has been suggested[8] that a deficiency of the B complex is common in both pernicious anemia and sprue and often explains many of the findings which are observed in these diseases. It is well known that there is no constant relationship between the degree of anemia and the severity of the above-mentioned symptoms. Although the administration of the B complex vitamins has no hematologic effect, neurologic lesions in experimental animals can be

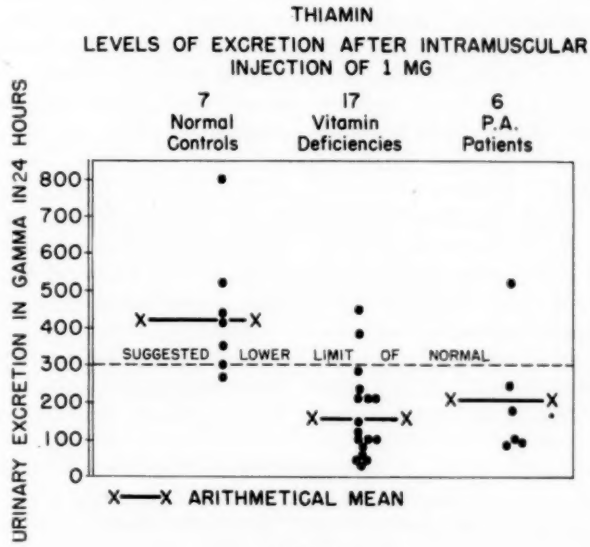

FIG. 6

prevented by the use of these substances.[9] The favorable influence of nicotinic acid on glossitis without alteration of the blood picture also has been noted.[10] Hansen[11] reports a case of a 39 year old white male with sprue who responded satisfactorily to intramuscular liver therapy. The patient later developed classical signs of pellagra while receiving the liver extract. These subsided promptly after the administration of nicotinic acid, supporting the belief that refined liver preparations may not contain adequate amounts of pellagra-preventive substances.

The present study was undertaken in an effort to determine the possible coexistence of an underlying B complex deficiency in patients having pernicious anemia. A study of the table and charts shows how closely the vitamin levels in the group having a B complex deficiency parallel those of the pernicious anemia patients. There was no apparent correlation between the degree of anemia and the vitamin levels in either group of patients. As might be expected, the levels of vitamin A and carotene in general reflected the nutrition of the group, and although lower than those of controls, were within normal limits. This is in striking contrast to studies on sprue, previously reported, in which a profound depletion of vitamin A and carotene in the plasma was found.[12] The blood levels of vitamin C were essentially normal.

Although the group studied is small, the definite lowering of nicotinic acid, thiamin, and riboflavin levels in these patients cannot be overlooked. One is impressed by the similarity of the distribution of the values in the pernicious anemia group to those in the vitamin deficiency patients. Therefore, the occasional reports of improvement of oral lesions on B complex therapy alone is not surprising. Complete recovery after liver therapy may be explained by the presence of sufficient accessory factors in the preparation used as well as by the well-known stimulating effect of liver upon the appetite with a resulting increase in dietary intake. The results of this study would justify the administration of B complex vitamins to patients having pernicious anemia if liver extract alone fails to produce a satisfactory response.

Conclusion. The laboratory determinations of vitamin levels of 8 patients having pernicious anemia show a coëxisting deficiency of the B complex vitamins.

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THE USE OF A HISTAMINE ANTAGONIST, BETA–DIMETHYLAMINETHYL BENZHYDRYL ETHER HYDROCHLORIDE, IN ALLERGIC DISEASE*

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Considerable evidence has accumulated in recent years emphasizing the rôle of histamine in anaphylactic and allergic reactions. In a review of the subject, Dragstedt[3] points out that while histamine may not account for all the manifestations of anaphylaxis, its relation to the reaction is given strong support by several established facts. It may mimic many of the important anaphylactic reactions; it is present in the tissues where such reactions take place; and its release in association with anaphylaxis has been conclusively demonstrated. In the field of human hypersensitivity the evidence involving histamine has been less conclusive. The similarity between the results of histamine injection and cold allergy was noted by Horton, Brown and Roth.[5] Rose and Browne[10] found that the histamine content of the blood of normal individuals showed great fluctuations in contrast to relatively constant levels in normal subjects. Elevated blood histamine in allergic diseases has been reported by some investigators,[1] while others[9] have failed to find any significant correlation between allergy and histamine content of the blood. Katz[6] has recently shown that histamine is released from human skin on contact of allergens in sensitive individuals. The search for a substance that counteracts histamine has therefore offered a hopeful approach to the non-specific treatment of allergic disease. Recently a group of synthetic benzhydryl alkaline ethers have been investigated and found to be markedly effective in preventing histamine and anaphylactic shock in guinea pigs.[7,8] The most effective member of this group is beta-dimethylaminethyl benzhydryl ether hydrochloride. Its marked histamine antagonism, inhibition of anaphylaxis, and relatively low toxicity have suggested its trial in the allergic conditions commonly encountered in everyday practice.

A group of 47 patients with a variety of allergic complaints including asthma, seasonal hay fever, urticaria, angioneurotic edema, eczema and allergic headache, were given the drug orally in doses of 50 to 600 mg. daily for varying periods of time.

The patients in the series were subjected to complete allergic studies, including skin tests. They were to some degree selected in that the drug was administered in instances where the specific etiology could not be determined; in cases where specific treatment had not advanced far enough to become effective in preventing symptoms; or where the usual measures at our disposal had failed to produce the desired results.

Urticaria and Angioneurotic Edema. Urticaria and angioneurotic edema were studied; 2 were of the acute variety, while 11 had chronic or recurrent symptoms of 1 month to 30 years duration, permitting a more accurate evaluation of the drug. Acute urticaria tends to improve spontaneously, and any results obtained in such cases must be judged in this light. All patients in the present study received some degree of improvement, ranging from the relief of pruritus to complete disappearance of lesions shortly after the first dose of 50 mg. The effect of the drug was noted within 1 hour and lasted in most instances from 2 to 12 hours, with a gradual return of symptoms when discontinued. Curtis and Owens[2] also reported improvement in a series of 18 cases of urticaria with recurrence of lesions upon discontinuance. Dosage in our study was found to be an individual problem. From 50 to 200 mg. daily was required to maintain results in the majority. In 1 case,

* The drug (Benadryl) for this investigation was supplied by Parke Davis & Co

however, 600 mg. was necessary to insure comfort and complete relief of symptoms. An effort was made to determine the etiologic factors in each case, and the drug was frequently discontinued to learn the effect of trial diets. When symptoms recurred, or when they were reproduced by an offending food, the drug was able to control the urticaria promptly. In this manner, considerable information regarding the etiology of the urticaria was obtained with a minimum of discomfort to the patient. Where the cause of the urticaria could not be determined, the minimum amount of the substance necessary to control symptoms was ascertained. A tendency to develop tolerance was noted when administered over long periods of time. As a palliative measure in these conditions, it appears to be the most effective agent yet encountered. It should not, however, be used to the exclusion of allergic investigation necessary to determine the underlying factors of each case. For the patient to continue to remain well, it is extremely important to determine the causative factors and thus eliminate the offenders.

Two cases (E. M. and H. L.) received only partial relief from symptoms when on either the diet or the drug. Symptoms were much less on dietary management, but on combined treatment, complete relief was obtained. Patient R. D., a 58 year old male, developed urticaria, polyarthritis and a severe generalized edema of the entire body after administration of 350,000 units of penicillin for a corneal ulcer. During the 1st week of symptoms, general medical measures, including ephedrine, adrenalin and large doses of vitamin C, were instituted, without results. He was then placed on the experimental drug in doses of 50 mg. every 2 hours. The urticaria and pruritus subsided following the initial dose, and within 24 hours the edema and all lesions had completely vanished. No change was noted in the polyarthritis. An attempt was made to reduce the dosage after a few days, but when given less often than 3 times daily (150 mg.) the urticaria recurred. This was immediately controlled with a single dose of 50 mg. At the time of this paper the patient still required 150 mg. daily.

Atopic Eczema. The effect of the drug on 4 patients with atopic eczema of long duration was observed. The most significant occurrence was a marked reduction of pruritus in 2 of these cases. This was accomplished with very small doses. No definite effect on the lesions themselves were noted, even when larger amounts were used. In 1 patient (D. D.) the lesions were thought to improve when the drug was first administered. Later it was found that symptoms fluctuated with the ingestion of certain foods. Good results have since been obtained by dietary measures alone. Another case (B. H.) had asthma in addition to eczema, but no effect on the former was noted during the course of treatment. The chief attribute of the drug in such cases seems to be its effect on pruritus.

TABLE 1.—CASES STUDIED

Diagnosis	No.
Urticaria and angioneurotic edema	13
Eczema (atopic)	4
Asthma (non-seasonal)	9
Hay fever (ragweed)	19
Allergic headache	2
Total	47

Asthma. No definite beneficial effect could be attributed to the drug in the 9 cases of asthma under consideration. In patient T. T., female, the condition was recurrent and there were definite psychogenic elements involved. Early in treatment she claimed relief for 3 hours after taking 50 mg. at bedtime. Later, however, she received no benefit whatsoever

TABLE 2.—URTICARIA AND ANGIONEUROTIC EDEMA

Beta-dimethylaminethyl benzhydryl ether hydrochloride

Case	Age	Sex	Duration and type	Other allergy	Daily dose (mg.)	Start	End	Side-effects	Results	Remarks
B. G.	41	F	3 mos. Chronic	..	50–150	3/30	5/11	Dizziness; GI upset	Improved	Small doses did not help; definite impr. with large doses; unable to cont. because of side-effects
J. G.	35	F	30 yrs. Recurrent	VR	50	4/27	5/4	....	Improved	Immed. response; remained symp.-free on diet after stopping drug; compl. control by elim. of food offenders
L. G.	50	M	4 mos. Chronic	..	50–200	5/4	5/23	....	Itching relieved; no effect on urticaria	
M. K.	20	M	1 yr. Recurrent	M and HF	150–50	6/27	7/3	....	Improved	Compl. relief after 1 dose; symp. did not recur after drug was elim.
M. K.	42	M	1 mo. Chronic	HF	150–50	4/2	4/14	Weakness	Improved	Immed. impr. on 150 mg.; reduced to 50 mg. after 5 days; aggravation from offending foods when on low dosage; milk main offender
R. B.	27	F	10 days Acute	..	50–100	2/16	4/14	Drowsiness	Improved	Relief of symp. 30 min. after dose; lasting 10 to 12 hours; recurrence of symp. several times when drug removed
W. P.	57	M	5 yrs. Recurrent	VR	100	5/14	6/30	....	Improved	Relief in 30 min. on 100 mg. p.r.n.; also noted some impr. in nasal symp. on taking 1 in A.M.; aspirin sensitivity later revealed as cause
M. A.	23	F	6 mos. Chronic	HF	50–150	1/23	4/3	....	Improved	Immed. relief on drug; good on smaller dose, but symp. recurred if discontinued; now takes 1 p.r.n.; food and emotions have definite influence
E. M.	28	F	2 yrs. Chronic	..	50	2/7	7/25	....	Improved	Incomplete relief on diet; Mechanical factors also play a rôle; complete relief from 50 mg. when on diet; both are necessary
R. W.	13	M	4 yrs. Recurrent	..	50	7/13	7/27	....	Improved	Definite impr. on 50 mg., but incomplete until food offenders eliminated; now no hives without drug, when on diet
H. L.	42	F	2 yrs. Recurrent	..	50	7/30	8/11	Fatigue	Improved	Less urticaria with drug; complete relief when used with food elim.
R. D.	58	M	2 wks. Acute	..	150–600	8/4	8/18	....	Improved	Urticaria and severe generalized edema after penicillin; urticaria subsided soon after initial dose; edema was gone in 24 hours; patient had to be maintained on 150 mg. to remain symptom-free; polyarthritis developed when urticaria impr. and not benefited by drug
A. F.	42	F	6 wks. Chronic	..	150	7/27	8/18	Drowsy	Improved	Immed. relief with drug; recurrence when discon.; impr. with food elim.

VR—vasomotor rhinitis. HF—hay fever M—migraine.

from the same or increased dosage. Her improvement coincided with the alleviation of emotional stress incident to family affairs. Another case, A. V., started with 50 mg. doses 3 times daily showing no improvement until 400 mg. were reached, but remained well after the drug was discontinued. Previous episodes in this patient seemed to run a similar course without any symptomatic medication. It is therefore doubtful whether there occurred any real therapeutic effect in any of these cases.

Hay Fever. Nineteen cases of ragweed hay fever were treated with the drug during the 1944 ragweed season. Among the patients in this series were some who had received little or no pollen treatment, and others who despite pollen therapy still had sufficient symptoms to justify other

TABLE 3.—ATOPIC ECZEMA

Case	Age	Sex	Duration	Other allergy	Etiology	Beta-dimethylaminethyl benzhydryl ether hydrochloride: Daily dose (mg.)	Start	End	Side-effects	Results	Remarks
L. B.	46	F	2 yrs.	..	P, M	50–200	5/26	6/15	Dizziness	0	Symptoms from spring to cold weather; some relief from desensitization to pollens and molds
D. D.	14	M	13 yrs.	HF and A	F	50–150	5/12	6/19	0	0	Impr. at first, but later had no effect
B. H.	29	F	13 yrs.	A	D, F	50–150	6/6	6/16	0	Relief from pruritus	Developed attack of asthma during course o. drug
G. S.	39	F	4 yrs.	..	F	50–150	3/30	4/27	0	Relief from pruritus	No change in skin condition

P—pollen. M—molds. D—dust. F—food.

TABLE 4.—ASTHMA

Case	Age	Sex	Duration	Other allergy	Skin tests	Beta-dimethylaminethyl benzhydryl ether hydrochloride: Daily dose (mg.)	Start	End	Side-effects	Results	Remarks
H. B.	46	M	7 yrs. Recur.	U and HF	E, P, F	50–100	5/15	5/30	0	0	Impr. after sufficient spec. desensitization
H. B.	49	F	17 yrs. Recur.	VR and GI	D, E, F, P	50	6/1	6/4	Weakness	0	Given for acute episode since, controlled by diet and desensitiz.
E. F.	21	F	15 yrs. Chr.	GI and VR	F, P	50	6/2	..	Faintness	...	Unable to continue because of side-effects
H. G.	59	M	2 yrs. Recur.	...	F, D	50	5/15	6/5	0	0	Given for acute exacerbation
F. K.	51	M	12 yrs. Recur.	...	P, D, E	150	5/18	6/4	0	0	Given for acute exacerbation
T. P.	50	F	10 yrs. Chr.	...	F, D	50	5/21	7/6	0	?	Given at bedtime when sympt. are usually present; felt that it helped at first, but later no effect
L. S.	15	F	1 yr. Recur.	HF	P, M	150	8/23	9/5	0	0	
R. W.	55	F	20 yrs. Chr.	HF	D, P, M	150	9/21	9/25	Weakness Drowsiness	0	
P. V.	20	F	3 yrs. Recur.	VR	F, D, P, M	100–400	7/25	8/1	0	Quest. impr.	Started on 100 mg. daily, incr. to 100 mg. 4 times daily; on 4th day remained well when drug was discontinued; patient moved away; no follow-up

U—urticaria. HF—hay fever. VR—vasomotor rhinitis. GI—gastro-intestinal allergy. A—asthma.
E—epidermal. P—pollens. D—dust. F—foods. M—molds.

measures. Symptoms were correlated with the daily pollen count, atmospheric conditions, and comparison with an adequate number of control patients in order to properly evaluate the effect of the drug. As pointed out in a recent hay fever study,[4] failure to employ these considerations have led to erroneous conclusions,

TABLE 5.—HAY FEVER

					Beta-dimethylaminethyl benzhydryl ether hydrochloride					
Case	Age	Sex	Duration	Status of pollen treatment 1944	Daily dose (mg.)	Start	End	Side-effects	Results	Remarks
D. D.	28	M	6 yrs.	I	150	8/23	9/1	Dizziness	Rhinitis; sl. impr. for short time	Symp. continued to fluctuate with pollen count
G. B.	65	F	13 yrs.	I	150	8/29	9/8	GI upset	0	Symp. fluctuated with pollen count
S. F.	35	F	7 yrs.	I	150	8/23	9/5	0	0	
M. F.	31	F	2 yrs.	I	150	8/28	9/20	0	0	Had asthma all through season; also mold sensitive
S. G.	12	F	5 yrs.	I	150	8/28	9/12	Dizziness	0	Food sensitivity present
S. G.	25	M	10 yrs.	I	150	9/11	9/19	0	0	
E. H.	46	F	8 yrs.	I	150	8/30	9/11	GI upset	0	
A. K.	30	M	15 yrs.	I	150	8/22	8/29	0	0	
M. L.	12	F	3 yrs.	I	50	9/6	9/13	0	0	Symp. alleviated when pollen count dropped
H. R.	33	M	10 yrs.	A	150	8/30	9/6	Fatigue	0	Symptoms fluctuated with pollen count and weather conditions; dust sensitive
F. S.	6	F	5 yrs.	A	150	8/11	9/2	0	0	
D. S.	15	F	4 yrs.	I	150	9/1	9/8	0	0	
S. S.	10	F	3 yrs.	A	150	9/8	9/15	Drowsiness	0	
A. V.	26	F	6 yrs.	I	150	8/30	9/13	0	0	
B. W.	23	F	6 yrs.	I	150	8/30	9/8	G.I. upset, drowsiness	+	Relief of symptoms day after drug started; remained good, but was forced to discontinue drug because of side effects; hay fever was minimal remainder of season without drug
W. T.	34	M	10 yrs.		150	8/30	9/8	0	+	Continued having symptoms but to a lesser degree; pollen count was dropping, no symptoms after drug was discontinued
P. H.	28	F	10 yrs.	N	50 50	5/18 7/2	5/21 7/7	0 0	0 0	
L. K.	36	M	3 yrs.	N	150	9/6	9/9	0	0	
R. S.	32	M	10 yrs.	N	150	9/6	9/30	0	Sl. impr. of rhinitis	Symp. fluctuated with pollen count

A—adequate. I—inadequate. N—none.

TABLE 6.—ALLERGIC HEADACHES

					Beta-dimethylaminethyl benzhydryl ether hydrochloride					
Case	Age	Sex	Duration	Other allergy	Daily dose (mg.)	Start	End	Side-effects	Results	Remarks
J. E.	43	M	1 yr.	VR	50	6/2	6/8	0	0	Has cont. headaches; gets definite relief from intradermal histamine injections
A. P.	27	F	15 yrs. Recur.	...	50	5/18	5/25	0	0	Well controlled for most part with dietary régime; tried drug when she went off diet; used on 4 occa.; 3 times no relief; once questionable

VR—vasomotor rhinitis.

as to the efficacy of numerous methods for the treatment of hay fever. Two patients received 50 mg. daily, while 17 were given 150 mg. daily in 3 divided doses. In some instances, improvement was observed which coincided with drops in the pollen count, and similarly observed in control subjects. Two patients (B. W. and W. T.) reported definite improvement shortly after the drug was started. Treatment was discontinued after a period of 8 days in both cases to determine whether symptoms would recur. In neither did symptoms appear during the remainder of the season. This occurred, however, during a decreasing pollen content of the air, making any definite evaluation of results in these 2 instances impossible. Patients B. D. and R. S. reported slight, but temporary improvement of their rhinitis, but one could not tolerate the drug. Both patients received similar relief of symptoms upon ingestion of $\frac{1}{150}$ gr. doses of atropine. The remaining 15 patients failed to note any benefit that could be directly attributed to the drug.

Allergic Headache. The drug was tried in 2 cases of headache which were thought to be of allergic origin. Neither case obtained relief when given for acute headache. Both have subsequently been controlled by dietary measures.

Toxicity. Of 47 patients in this series, 16 complained of some side-effects. The most common symptoms attributed to the medication were drowsiness, dizziness, weakness, faintness, fatigue and gastrointestinal upsets. In 3 patients it was found necessary to discontinue the drug because of the side-effects. In a few other cases dosage was necessarily restricted by these annoying symptoms. The majority, however, were able to tolerate the medication without undue reactions.

Comment. Beta - dimethylaminethyl benzhydryl ether hydrochloride appears to be an extremely effective agent in the palliative treatment of urticaria and angioneurotic edema. Its effect is rather short, and symptoms in chronic cases tend to recur after it is discontinued. In the cases under study in this report, it served as an excellent aid in controlling discomfort while the necessary procedure for the determination of etiologic factors were carried out. Its use in chronic cases over long periods of time without attention to possible underlying allergic etiology does not seem justified, especially in view of occasional side-effects, and increased tolerance on extended use.

The drug also has some effect in reducing the pruritus incident to atopic dermatitis. It may likewise be effective in pruritic conditions of other origin.

Experimental evidence in guinea pigs indicated that this substance is one of the most potent histamine antagonists yet found. The good results observed in urticaria, and the failure to benefit other atopic conditions, suggest the possibility that histamine may play a greater rôle in urticaria than in asthma, hay fever, and other allergic manifestations. There is also the possibility that the antihistamine substance may be necessary in larger amounts to produce effects in conditions other than urticaria. Routes of administration other than oral may perhaps be necessary to produce the desired results in other allergic conditions.

Summary. 1. Beta-dimethylaminethyl benzhydryl ether hydrochloride, a potent histamine antagonist in guinea pigs, was administered to 47 patients with various allergic complaints.

2. Marked benefit, which continued in most cases as long as the drug was administered, was noted in urticaria. Relief of pruritus was noted in some cases of atopic eczema. No benefit was apparent from its use in asthma, hay fever, or allergic headache.

3. The results suggest that histamine may be a more important factor in urticaria than in other allergic conditions.

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CAPILLARY MICROSCOPY

WITH SPECIAL REFERENCE TO CAPILLARY PETECHIÆ

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THE actual observation of nail-bed capillaries by microscopy (capillaroscopy) is disarmingly easy. The technique has often been described. The key to the literature and basic work will be found in the works of Mueller[4,5] (1922, 1937 and 1939), and in the papers of Wright and Duryee[6] (1933), Leader[3] (1932) and Callander[1] (1940). A special capillary microscope is not essential. Excellent observation is possible with any good laboratory microscope, although a binocular instrument is preferable. Using a standard binocular microscope I have examined the finger nail-beds of over 1000 patients (including many out-patients), without any special preparation and without scrubbing the nails. I observed at least 2 fingers, but usually 3 fingers, and often 4 fingers, of each hand. The patients' ages ranged from 5 to 87 years. The capillary pattern in any particular healthy adult is usually strikingly constant—for example, my own has not varied significantly in over 6 years of almost weekly observation. Nevertheless there is often much variation between the capillaries in a single nail-bed, and sometimes great variation from finger to finger, and between the fingers of both hands. Yet unwarranted inferences have been drawn from variations well within normal. Various specialists have examined the nail-beds of a few dozen patients suffering from a particular disease, and arrived at conclusions without adequate controls in normal persons and patients with other diseases.

Capillary Petechiæ. I first became interested in capillaroscopy as an aid in the study of purpura.[2] Observations showed that capillary petechiæ were common. Thus there were 100 patients with capillary petechiæ in 533 consecutive patients whose nail-beds were examined microscopically. This incidence (18.4%) is unduly high, because on a basis of previous experience, patients likely to show abnormalities and petechiæ were specially selected. Nevertheless, most types of patient using the medical division of a general hospital were examined contemporaneously, and healthy persons in addition. It is possible to give a general picture of the incidence of these petechiæ.

The patients showing capillary petechiæ have been divided into 3 groups (see table). Group 1 comprises a mixed collection of various types of cases, in each of which type capillary petechiæ were seen once only, or not at all. Among 262 such cases, which included various diseases of the heart, lung, kidney, nervous system, blood and skin, petechiæ were seen 23 times (an incidence of 8.8%). In Group 2 are included diseases in some of which the frequency of capillary petechiæ may prove significant, but where much more evidence is wanted. Group 3 comprises the conditions in which petechiæ were most frequent. They were scurvy, bronchiectasis, arteriosclerosis, purpura simplex, Raynaud's disease and clubbed fingers. Separate figures for clubbed fingers are not given in the table because all patients with clubbing were listed according to their disease. Thus of the 6 patients with bronchiectasis and capillary petechiæ, 5 had clubbed fingers, as had both patients with Fallot's tetralogy, and 2 of the 3 patients with syphilis. Of the 533 patients considered, 23 had clubbed fingers, and 11 of these had capillary petechiæ.

Morphology. The appearance of the petechiæ is shown in the figures, in about

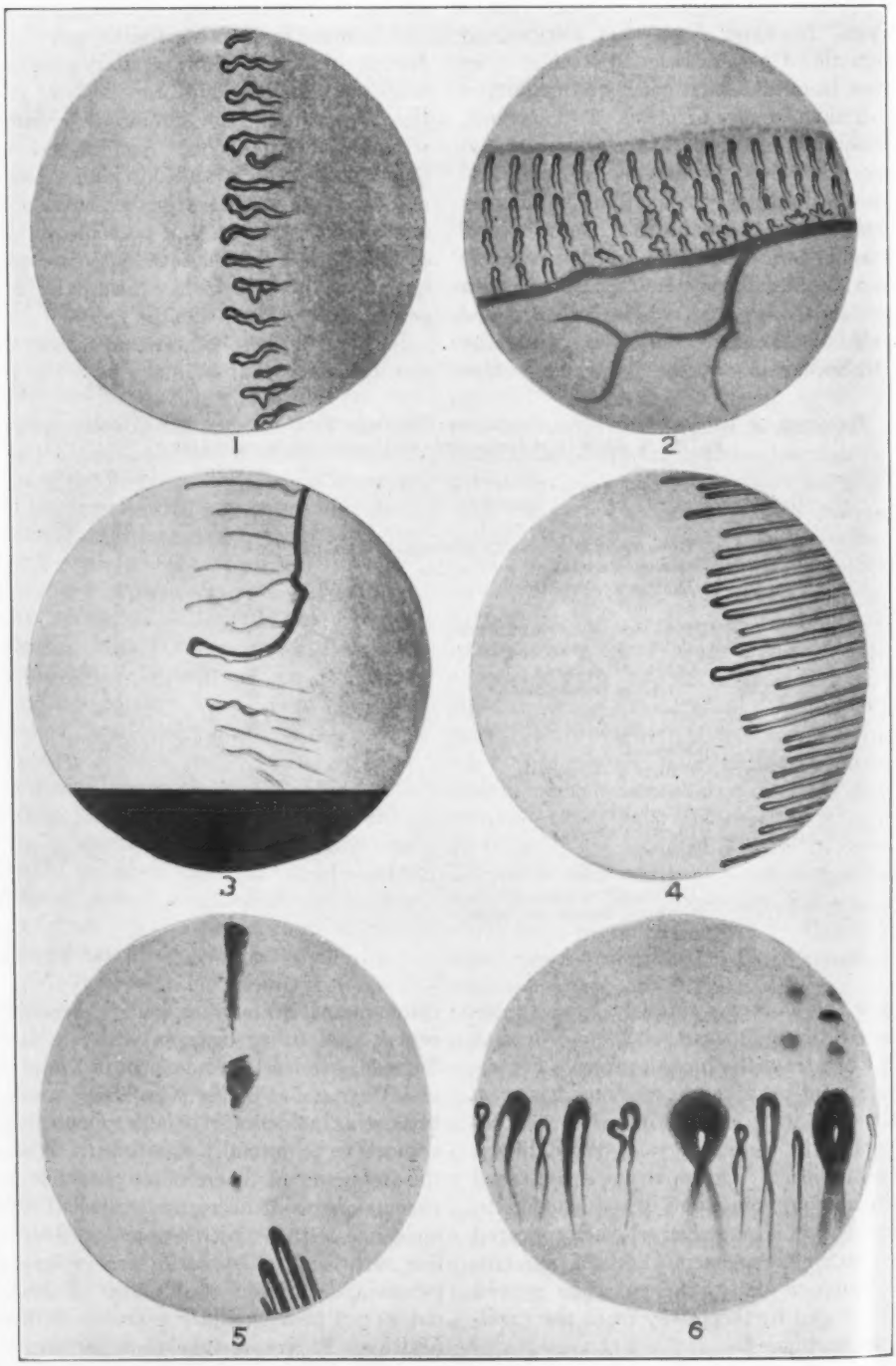
the order of frequency of the various types. No type of petechia was pathognomonic of any disease. Petechiæ often arose from abnormal congested capillaries with sluggishness or stasis of blood flow. Petechiæ seemed to arise nearly always from the loop joining the arterial and venous limbs of the capillary, and very rarely from these limbs. Occasionally, petechiæ were seen actually separating from the capillary. In 2 patients, after such separation, the capillary re-attached itself to the petechia, and then the petechia again became detached. Once I saw the loop of a capillary (grossly distended through stasis of blood) become detached from the limbs to form a hemorrhage, and on several occasions a portion of such a loop became detached. Occasionally the petechia was shaped like a capillary cast. Petechiæ when first shed were obvious collections of red blood cells, later they became darker, and appeared as a rather homogeneous mass of pigment. If examined daily, the petechiæ moved further and further away from the capillaries, and faded.

ANALYSIS OF 100 PATIENTS WITH CAPILLARY PETECHIÆ FOUND AMONG 533 CONSECUTIVE PATIENTS RECENTLY EXAMINED BY CAPILLARY MICROSCOPY

Group	Disease	No. cases examined	No. with capillary petechiæ
1	Miscellaneous (see text)	262	23 (8.8%)
2	Rheumatic fever (acute or chronic)	54	9
	Diabetes mellitus	26	4
	Epilepsy (idiopathic)	22	3
	Syphilis	13	3
	Schönlein-Henoch purpura	11	3
	Pulmonary tuberculosis	10	3
	Osteo-arthritis	8	2 (23.1%)
	Infective polyarthritis	5	2
	Gout	4	2
	Virus hepatitis	7	2
	Malaria	4	2
	Pituitary diseases	3	2
	Fallot's tetralogy	2	2
3	Arteriosclerosis	39	11
	Purpura simplex	31	10
	Scurvy	11	7 (27.4%)
	Bronchiectasis	14	6
	Raynaud's disease	7	4
	Clubbed fingers (see text)		
	Total	533	100 (18.4%)

Discussion. In some patients capillary petechiæ and skin petechiæ occur simultaneously. In others, skin petechiæ precede or follow the findings of capillary petechiæ. But many patients show capillary petechiæ when no skin petechiæ were seen at any time. (Compare the similar discrepancy when retinal or mucuous membrane hemorrhages are present and skin hemorrhages absent, or when visceral petechiæ are seen at necropsy without skin petechiæ.) Occasionally the skin has presented a widespread massive petechial eruption, but petechiæ were absent at the nail-bed.

In spite of these tantalizing discrepancies, it is clear that capillary petechiæ are common, particularly in scurvy, bronchiectasis and other diseases with clubbed fingers, arteriosclerosis, purpura simplex and Raynaud's disease. In these conditions, the incidence of capillary petechiæ appears to be specially significant. When the frequency of macroscopic petechiæ in various organs at necropsy is recalled, the incidence of these microscopic petechiæ is less surprising. Capillaries are prone to petechiæ formation in disease. I have not as yet seen capillary petechiæ in the healthy. It is possible that for every petechia visible to the naked eye in any patient, there are many microscopic pete-



KEY TO FIGURES

All capillaries were seen at × 60 and drawn to scale.

FIGS. 1 to 4.—Types of normal capillaries.

FIGS. 5 to 11.—Types of petechiæ.

FIG. 11.—Petechiæ in formation.

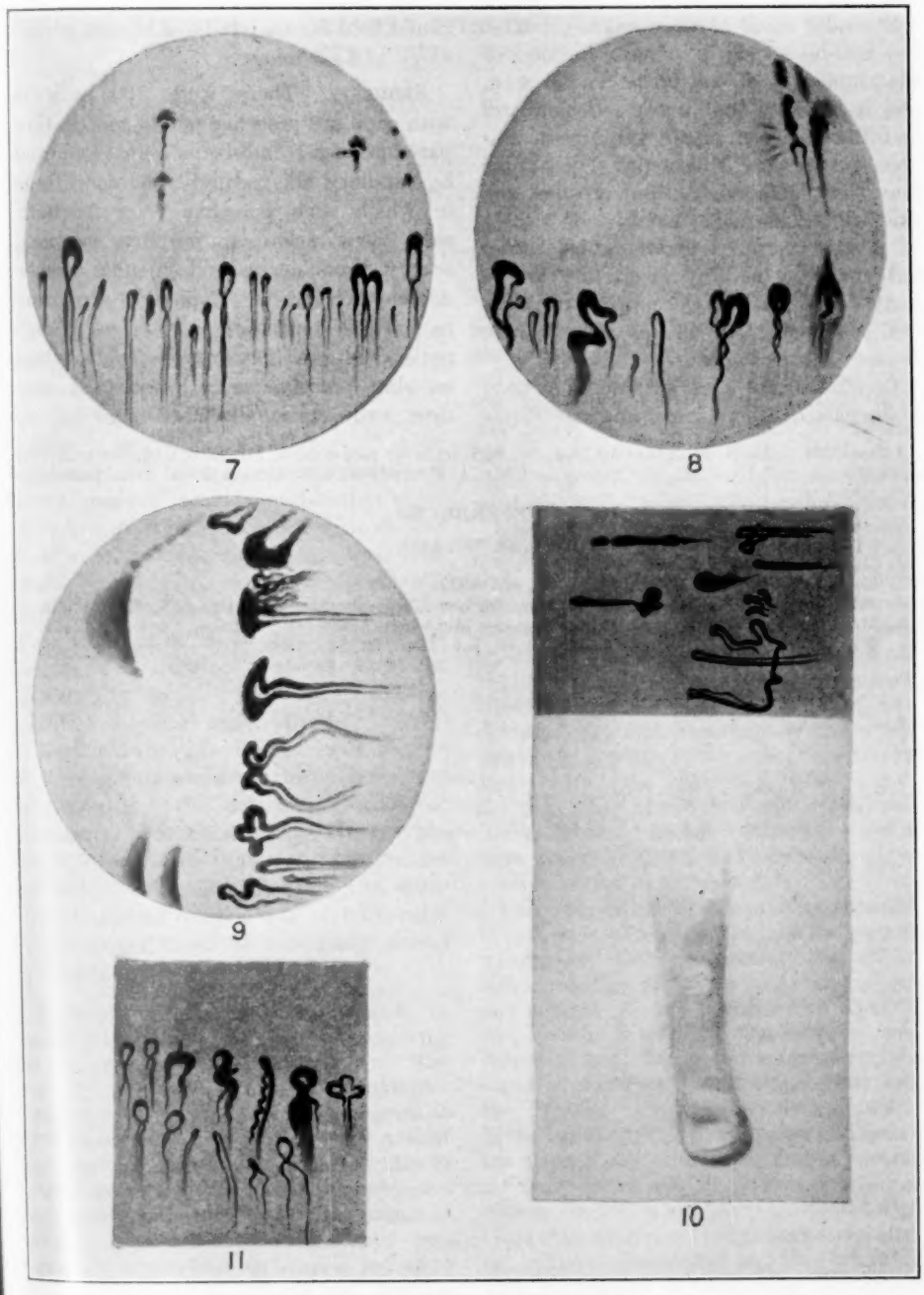


FIG. 5.—Patient with pulmonary tuberculosis.
FIG. 6.—Patient with recurrent skin purpura following influenza.
FIG. 7.—Patient with chronic rheumatic carditis.
FIGS. 8, 9 and 11.—Patients with Raynaud's disease.
FIG. 10.—Patient with Fallot's tetralogy.

chiæ formed. Nail-bed capillaroscopy will render some of these visible, but at the nail-beds only a minute fraction of the capillaries of the body can be seen, and it is likely that many petechiæ will be formed at other sites. In purpura simplex, only a small fraction of skin surface may show hemorrhage, and most of the skin will be normal. Yet in 1 of 3 cases of purpura simplex, examination (often but once) of the tiny proportion of the body's capillaries at the nail-bed, will reveal petechiæ. And the incidence in some diseases is even higher.

Capillaroscopy gives no trouble or pain to the patient, and is gratifying and stimulating to the observer. Here is an additional field for the study of human physiology and pathology.

Summary. There were 100 patients with capillary petechiæ in 533 consecutive patients whose nail-beds were examined by capillary microscopy. The conditions in which such petechiæ were frequent were arteriosclerosis, purpura simplex, scurvy, bronchiectasis, Raynaud's disease and clubbed fingers. Capillaries are prone to petechiæ formation in disease. Many patients showed capillary petechiæ when no skin petechiæ were present at any time, and occasionally *vice-versa*.

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CLINICAL AND LABORATORY STUDIES OF LIVER FUNCTION IN THERAPEUTIC MALARIA*

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The exact status of the pathologic changes occurring in the liver during malaria infections is not well understood, but the topic has received increasing attention in recent investigations.[1,3,4,6] Our interest in this subject was aroused when several cases of jaundice occurred as complications of therapeutic malaria at the Neurosyphilis Center of this general hospital. Furthermore, in view of the current emphasis on dietary constituents as a protection against or treatment for hepatitis, it was decided that an excellent opportunity to evaluate the protective qualities of special diets existed.

A critical study to determine the degree of liver involvement during malaria was facilitated by the active utilization of therapeutic infections on the Neurosyphilis Center. The patients were under well-controlled conditions, and the duration of clinical malaria was often considerably longer than the customary period of treatment for neurosyphilis.

Material and Method. The patients included in this study were all soldiers requiring malaria therapy for neurosyphilis. The majority were young men with asymptomatic neurosyphilis, and were otherwise in good general health. Almost every patient had previously received at least 6 months of mapharsen and bismuth chemotherapy prescribed by the Army in the treatment of syphilis. Malaria inoculations were performed by a variety of techniques, including mosquito application, intradermal inoculation, and intravenous inoculations with varying parasite dosages.

The status of the liver was followed with recorded observations, both clinical and laboratory, prior to the onset of malaria paroxysms, periodically during the paroxysms, and following the therapeutic course until the patient's discharge from the hospital.

Clinical observations were made very frequently. During the actual febrile course, the patients were seen at least twice daily by one of the medical officers in attendance. Formal rounds were made daily by at least one of us. Quantitative observations on the following details were made on each patient. General condition: the presence of any digestive symptoms, particularly the condition of the appetite or the presence of nausea and vomiting; the degree of jaundice, if any; the palpability of the liver and spleen; and the presence of edema. Patients' weights were closely followed with observations recorded once or twice weekly.

Complete laboratory data were obtained in each case. Those pertinent to the present investigation were: the hemoglobin; icterus index; van den Bergh reaction, both direct and indirect; plasma proteins with albumin and globulin partition; oral hippuric acid excretion test; the prothrombin time; the cephalin cholesterol flocculation test; and the urinary urobilinogen determination. These laboratory tests were performed before the onset of clinical malaria, and at varying intervals during and after the active febrile course. In the case of those inoculated with vivax malaria, repeat studies were made after the initial 10 paroxysms, and then following each subsequent 5 paroxysms. In those

* All laboratory tests were performed under the direct supervision of DeWitt F. Mullins, Capt., M.C., Chief of Laboratory Service.

† Major Glenn died after this study was completed.

with quartan malaria, the determinations were made after the initial 5 paroxysms, and then after each subsequent 5 paroxysms. In both instances (vivax and quartan), at least 1 follow-up test was performed approximately 10 days after the institution of atabrine therapy. In some cases, 2 and 3 follow-up tests, at different intervals, were performed if the patient was hospitalized long enough.

The patients in the study were divided into 3 groups, designated Groups A, B, and C. There was no selection of patients for this grouping, for they were placed in a consecutive group as they were inoculated. The only exceptions were Negro patients, all of whom were inoculated with quartan malaria. Arbitrary group assignments were here made in order to equally distribute the smaller number of quartan-infected cases. There were 20 patients in each group—15 with vivax malaria and 5 with quartan malaria, making a total of 60 patients in the entire study.

Group A was the control group and received only the routine treatment with no additional medication. They were given the regular hospital diet, which averaged 335 gm. of carbohydrate, 115 gm. of protein, and 164 gm. of fat, for a total of 3300 calories daily. In this group, as well as in the other groups, meals were tolerated best on paroxysm-free days. On febrile days, patients definitely ate less, ingesting very little solid food and mostly liquids. This general digestive status was altered as noted by the specific observations in Table 2.

Patients in Group B were given, in addition to the regular diet as prescribed for Group A, additional protein intake in the form of more high protein foods. They were served with "protein cocktails," 3 times daily, each consisting of a glass of milk containing 1 egg and 1 tbs. of Brewer's Yeast Powder or Aminoids. If 3 cocktails were consumed daily, an extra protein intake of 45 gm. was provided. Group B patients were also given multivitamin capsules, 2, 3 times daily, each containing 2500 units vitamin A, 200 units vitamin D, 1 mg. thiamine chloride, 1.5 mg. riboflavin, 10 mg. nicotinic acid, and 37.5 mg. ascorbic acid. They also received crude liver extract injections (3 cc. each—assayed at 2 reticulogenic units per cc.) intramuscularly every other day.

Group C was on the same regimen as Group B, but received an additional 1000 cc. of intravenous glucose solution daily. This consisted of 5% glucose in saline and 10% glucose in distilled water on alternate days, thus providing each patient in Group C with an added average dose of 75 gm. of glucose intravenously daily during the course of the fever. 100 mg. of ascorbic acid and 50 mg. of thiamine chloride were added to each intravenous infusion. Both Groups B and C were allowed to raise their protein intake as they desired by taking additional Brewer's yeast or aminoids in peanut butter sandwiches, cereal, etc.

Results and Discussion. 1. CLINICAL OBSERVATIONS. Tables 1 and 2 summarize the clinical observations made on each patient in the 3 groups during their febrile period. As noted, there are 3 degrees of symptoms recorded: those definitely present (+), those definitely not present (0), and those in which the degree is recorded as questionable (?). It was found that this latter category had to be included, because in so many cases the symptoms were so slight or of such brief duration that opinions as to its definite presence varied between the different observers or even by the same observer at different times.

Table 1 shows the averages for the number of paroxysms reaching 104° F. or more; the number of hours of fever experienced above 103.6° F.; the lowest hemoglobin level; and the % of weight lost in each of the 3 groups, with separate averages for the vivax and quartan infections in each group. These criteria give some indication of the severity of the malaria infection in each group.

It is noted that in those cases receiving quartan malaria therapy, the average number of hours of fever above 103.6° F. is much greater than in the vivax group, even though the average number of paroxysms experienced with each species is about the same. This, of course, is to be expected from the type of fever plateaus commonly observed in quartan infections. A significant observation, illustrated by the averages listed in Table 1, is that the

malaria in Groups B and C was a more severe disease than in Group A. The average number of paroxysms and the average number of hours of fever at the established level in Groups B and C were definitely greater than in Group A; hemoglobin values fell to lower levels than in Group A, and weight loss was greater than in Group A.

with malaria therapy were about equal in all groups.[7] The patients in Group C seemed to remain in a somewhat better general condition, complaining less of weakness and fatigue during their paroxysms than did those in Groups A and B. This observation, however, was difficult to evaluate objectively and is, therefore, not included in the tables.

TABLE 1.—A COMPARISON OF AVERAGE VALUES FOR THE DURATION OF FEVER, LOWEST HEMOGLOBIN LEVEL, AND % WEIGHT LOSS IN GROUPS A, B, AND C

Average of:	No. paroxysms above 104° F.	No. hours fever 103.6° F.	Hemoglobin lowest level (gm.)	Weight loss (%)
Group A—Vivax	15.3	58.0	7.8	7.1
Quartan	8.8	53.8	9.0	8.5
Total	13.6	57.0	8.1	7.4
Group B—Vivax	17.7	63.4	7.9	7.7
Quartan	16.0	100.6	7.6	11.6
Total	17.2	72.7	7.8	8.7
Group C—Vivax	18.0	62.7	7.9	8.7
Quartan	15.2	95.4	7.5	10.6
Total	17.3	70.8	7.8	8.4

TABLE 2.—COMPARISON OF CLINICAL OBSERVATIONS DURING THE FEBRILE PERIOD IN GROUPS A, B, AND C

	Abdominal pain			Loss of appetite			Nausea			Vomiting			Jaundice			Liver			Spleen			Edema		
Degree:	+	?	0	+	?	0	+	?	0	+	?	0	+	?	0	+	?	0	+	?	0	+	?	0
Group A—																								
Vivax	3	0	12	12	2	0	7	4	3	2	7	5	7	1	6	8	2	5	9	2	4	3	0	11
Quartan	1	0	4	3	2	0	1	1	3	1	0	4	1	0	4	1	1	3	0	0	5	2	0	3
Total—No.	4	0	16	15	4	0	8	5	6	3	7	9	8	1	10	9	3	8	9	2	9	5	0	14
%	20	0	80	79	21	0	42	26	32	16	37	47	42	5	53	45	15	40	45	10	45	26	0	74
Group B—																								
Vivax	3	0	12	8	6	1	0	11	4	1	9	5	8	2	5	4	5	6	10	3	2	2	1	12
Quartan	0	0	5	2	3	0	0	1	4	0	0	5	1	1	3	3	0	2	2	0	3	1	0	4
Total—No.	3	0	17	10	9	1	0	12	8	1	9	10	9	3	8	7	5	8	12	3	5	3	1	16
%	15	0	85	30	45	5	0	60	40	5	45	50	45	15	40	35	25	40	60	15	25	15	5	80
Group C—																								
Vivax	3	0	12	10	5	0	4	6	5	5	4	6	9	0	6	1	3	11	11	1	3	5	0	10
Quartan	1	0	4	2	3	0	0	3	2	0	3	2	0	0	5	0	2	3	3	0	2	1	0	4
Total—No.	4	0	16	12	8	0	4	9	7	5	7	8	9	0	11	1	5	14	14	1	5	6	0	14
%	20	0	80	60	40	0	20	45	35	25	35	40	45	0	55	5	25	70	70	5	25	30	0	70

+ indicates the definite presence of the symptom or palpability of the organ at some time during the febrile course. ? indicates the minimal or questionable presence of the symptom or questionable palpability of the organ at some time during the febrile course. 0 indicates the absence of the symptom or non-palpability of the organ at any time during the febrile course.

Table 2 is a summary table showing the number of patients, subdivided into vivax and quartan cases in each group, with the various degrees of symptoms. There is no striking difference in the frequency of any of the observations in the 3 groups, except for the palpability of the liver. Complications of various types, other than liver dysfunction, occurring in connection

In both Groups B and C, the distaste for "protein cocktails" was uniform. It is difficult to mix a tasty preparation of a high protein supplement including any of the specially concentrated protein compounds. However, in 1 recorded period of observation of 1 month, the patients averaged 32 gm. of protein supplement daily from the "protein cocktails" alone.

There was no very striking difference in the incidence of abdominal pain and digestive symptoms in the 3 groups. Nausea and vomiting had to be separately considered, since certain patients did not experience nausea, but would vomit each time they attempted to eat. Nausea was of lesser severity in Group B than in either of the other groups, but this slight variation may have resulted from the small size of the groups involved. In view of the fact that the malaria infections in Groups B and C were definitely more severe than in Group A, the slightly lower frequency of digestive symptoms in these groups may have greater significance.

Clinical jaundice appeared about equally in all 3 groups, and was usually slight and limited to the sclerae. Jaundice was observed in a larger number of cases than indicated by the icterus index and van den Bergh determinations. This, of course, is not surprising, since it may have been observed as a transient phenomenon during the very frequent clinical observations, and yet have been missed in the chemical determinations made at less frequent intervals. Furthermore, observations of minimal jaundice may at times be quite confusing, particularly near the end of the febrile course. At that stage, because of the generalized pallor, slight degrees of jaundice are difficult to evaluate. In addition, some of the patients still had residual skin discoloration from long periods of suppressive atabrine therapy in overseas theatres. At any rate, there was no definite correlation between the clinical observation of jaundice and the palpability of the liver.

The most interesting clinical observation concerned the palpability of the liver. The number of patients with enlarged livers in Group C was definitely less than in either Groups A or B, as noted in Table 2. In only 1 case (5%) in Group C was the liver definitely palpable, as contrasted to 7 cases (35%) in Group B and 9 cases (45%) in Group A. On the other hand, in 14 cases (70%) in Group C, the liver was definitely not palpable during the entire course of malaria; whereas in Group B there were only 8 cases (40%) and in Group A 8 cases (40%) in which the liver was never palpable. However, there was no definite relation of digestive symptoms to the size of the liver. The difference in liver palpability was in contrast to the similar incidence of digestive symptoms in each group. It is, therefore, probable that digestive symptoms are not entirely either a cause or a result of liver enlargement.

Furthermore, the absence of liver enlargement in Group C is not due merely to the fact that this group had the highest calorie intake from the additional intravenous glucose, because the % weight loss in this group was similar to that in Group B and more than that in Group A. There is, therefore, the possibility that the addition of intravenous glucose in Group C's regimen had some relation to the observation that the liver was least often palpable in this group. If it is assumed that enlargement of the liver is *prima facie* evidence of some liver derangement, perhaps of fatty metamorphosis, one can conjecture that such involvement of the liver was least in Group C.

After the completion of each malaria course, the parasitemia was terminated with atabrine therapy consisting of 0.2 gm. of atabrine every 6 hours for 5 doses, followed by 0.1 gm. 3 times daily for 6 days—a total dose of 2.8 gm. of atabrine. After antimalarial therapy had been completed, most of the patients received 10 daily injections of mapharsen (0.06 gm. daily). In 6 cases (4 in Group A, 1 each in Groups B and C), the liver became definitely more enlarged after the mapharsen injections had been started. In 1 other case, in Group C, the liver became questionably palpable following atabrine therapy, even though this particular patient did not receive mapharsen.

In 270 cases of neurosyphilis treated with therapeutic malaria prior to the controlled study herein reported, the liver was found to be palpable in 24.7% at some time during the clinical course. This

less frequent enlargement can be explained only on the basis of infrequent and less careful examinations.

The spleen was palpably enlarged slightly more often in Groups B and C than in Group A. This may have been the result of the increased severity of malaria in these groups, as previously noted. The fact that the frequency of enlargement of the liver in the 3 groups varied in the opposite direction from the frequency of splenic enlargement supports the belief that the regimen, on which Group B and particularly Group C were placed, may have prevented liver enlargement.

various laboratory determinations in the 3 groups before, during, and after the febrile course of malaria. The average icterus index during the febrile period was slightly, though not significantly, higher in Group A than in Groups B and C. There were very few icterus index readings in the range of clinically discernible jaundice.

(*b*) *The van den Bergh Reaction.* The van den Bergh determinations were performed by the method of Goodrich and Gibson. Table 3, line B, shows that, again, determinations were very similar in the 3 groups.

TABLE 3.—LABORATORY DETERMINATIONS SHOWING THE RANGE AND AVERAGE OF THE 20 CASES IN EACH GROUP BEFORE, DURING, AND AFTER THE FEBRILE PERIOD

	Group A		Group B		Group C	
	Range	Average	Range	Average	Range	Average
Icterus index:						
Before	3.2-17.7	8.9	2.4-14.3	8.2	5.3-11.4	8.3
During	5.6-38.1	12.3	4.5-24.0	10.3	5.6-23.5	11.0
After	4.2-11.1	7.1	4.6-11.9	7.2	4.0-16.0	7.4
Indirect van den Bergh:						
Before	N*-1.95	0.75	N-1.44	0.65	N-1.7	0.60
During	N-3.80	1.25	N-4.00	1.06	N-2.95	1.16
After	N-0.81	0.27	N-0.90	0.28	N-0.71	0.26
Hippuric acid excretion:						
Before	2.0-4.8	3.6	1.0-4.7	3.5	1.8-5.2	3.4
During	1.2-5.4	3.1	0.2-5.3	3.1	0.9-5.2	3.0
After	0.1-5.3	2.4	0.2-4.5	2.2	0.1-5.2	2.5
Plasma proteins:						
Before	6.6-8.3	7.6	6.6-9.1	7.5	7.1-8.9	7.6
During	5.6-8.2	6.5	4.9-7.8	6.4	5.5-8.0	6.5
After	6.3-8.5	7.2	5.9-8.8	7.1	5.6-8.5	7.2
Albumin:						
Before	3.9-6.2	5.2	3.6-5.8	4.8	4.0-5.7	4.9
During	2.7-4.5	3.6	2.6-4.1	3.5	2.5-4.5	3.6
After	3.3-5.1	4.4	3.1-5.5	4.3	3.3-5.7	4.4
Globulin:						
Before	1.8-4.3	2.7	2.0-3.9	2.7	2.0-4.2	2.7
During	1.6-4.1	2.9	1.6-4.2	3.0	1.8-4.3	3.0
After	2.0-4.2	2.8	1.9-4.2	2.8	2.0-3.7	2.8

*N signifies normal bilirubin determination, but too low to read accurately. In computing the averages, this value was taken to be 0.2 mg. per 100 cc.

Edema was observed in about the same frequency in Groups A, B, and C. In all cases it was only minimal. In none of the edema cases were there any cardiac or urinary abnormalities to suggest either as an etiologic factor in the production of this complication.

2. LABORATORY STUDIES. (*a*) *Icterus Index.* Icterus index determinations were done by the method of Bernheim. Table 3 shows the ranges and averages of the

Some of the determinations for the indirect van den Bergh reaction in the period before the onset of fever were slightly high for the value considered to be normal. The same was true of several icterus index, cephalin cholesterol flocculation, and urinary urobilinogen determinations. The explanation for this may be that these laboratory studies were usually made after the inoculation of the patient with malaria parasites. Most of them, there-

fore, were studied during the pre-patent or incubation periods, during which time alterations to be observed during the febrile course may have already been initiated. At any rate, in none of the cases showing abnormal determinations during the pre-febrile period was there any clinical evidence of preëxisting liver disease. Furthermore, in all of these cases, subsequent determinations after the febrile period, and in some instances even during the febrile period, were within the normal range.

There was no apparent correlation between the average bilirubin determinations before, during, and after the febrile period and the palpability of the liver. In addition, there was no particular difference in the incidence of elevated blood bilirubin in patients with various degrees of liver enlargement (Table 4). However, elevated bilirubin beyond the manifest jaundice level (2.6 mg. per 100 cc. or icterus index above 18 units) occurred twice as frequently among patients with enlarged livers as in those without increased liver palpability (Table 4). It is felt, therefore, that hyperbilirubinemia was largely due to the hemolytic effect of malaria rather than to involvement of the liver. This is, perhaps, corroborated by the fact that most of the determinations showed only an indirect van den Bergh reaction.

The direct van den Bergh reaction was diphasic, immediate, or delayed direct in only a very few instances (7 in Group A, 4 in Group B, and 5 in Group C). In these cases, in which the direct van den Bergh reaction was other than negative, the highest icterus index and direct van den Bergh readings (above 1 mg. per 100 cc.) were uniformly encountered. From the reverse aspect, however, not all of the van den Bergh determinations above 1 mg. per 100 cc. were associated with diphasic or direct reactions. Table 5 shows that the diphasic van den Bergh reactions were most frequent in cases with liver enlargement. These observations indicate that hyperbilirubinemia was not entirely due to hemolysis, but in at least some instances to hepatocellular disease, particularly in those cases with manifest jaundice and enlargement of the liver.

A correlation of digestive symptoms with blood bilirubin levels in Table 6 shows that the incidence of definite loss of appetite increases with the bilirubin level. With values below 1 mg. per 100 cc. only 40% of patients had marked loss of appetite. In the range of latent jaundice, this increased to 72%, whereas with manifest jaundice (bilirubin levels above 2.6 mg. %), 91% of patients experienced definite anorexia. The variations of other digestive symptoms were not as pronounced.

TABLE 4.—INCIDENCE OF ELEVATED BILIRUBIN AND CHEMICAL MANIFEST JAUNDICE RANGE IN RELATION TO DEGREE OF LIVER ENLARGEMENT

Liver		Elevated bilirubin		Manifest jaundice	
		Bilirubin above 1 mg./100 cc. Icterus index above 12 units		Bilirubin above 2.6 mg./100 cc. Icterus index above 18 units	
Palpability of liver	No. cases	No.	%	No.	%
+	17	11	64.7	5	29.4
?	12	9	75.0	2	16.6
0	27	16	59.2	4	14.8

For explanation of +, ?, and 0, see Key, Table 2.

(c) *Plasma Proteins, Albumin and Globulin Partition.* The plasma protein determinations were done by the method of Howe (Kjeldahl). Table 3, lines D, E, and F, lists the range and averages of the plasma proteins and the albumin and globulin fractions in each of the 3 groups during the 3 periods of observation. No particular difference between the groups was apparent.

The total protein decreases during the febrile period, but returns almost to normal levels thereafter. Simultaneously with this decrease, there is a slightly

greater fall in the albumin fraction and a coincident slight rise in the globulin fraction. These changes in plasma proteins during the course of malaria have been previously described by Kopp and Solomon.[5]

(*d*) *Hippuric Acid Excretion Test.* The hippuric acid excretion test was performed by Quick's ether extraction and digestion method. No urine specimens were sent to the laboratory for determination if the test seemed incomplete in any way.

TABLE 5.—INCIDENCE OF DIPHASIC AND DIRECT VAN DEN BERGH REACTIONS DURING THE FEBRILE PERIOD RELATED TO VARIOUS DEGREES OF LIVER ENLARGEMENT

Liver		van den Bergh reactions					
		Diphasic		Direct immediate		Direct delayed	
Palpability of liver	No. cases	No.	%	No.	%	No.	%
+	17	5	29.4	1	5.9	1	5.9
?	13	1	7.7	1	7.7	1	7.7
0	30	3	10.0	1	3.3	2	6.7

For explanation of +, ?, and 0, see Key, Table 2.

TABLE 6.—INCIDENCE OF BLOOD BILIRUBIN LEVELS AND VAN DEN BERGH REACTIONS RELATED TO DIGESTIVE SYMPTOMS

	Blood bilirubin (mg./100 cc.)						van den Bergh reaction			
	Above 2.6 mg.		1-2.6 mg.		Below 1 mg.		Diphasic immediate or delayed direct		Indirect only	
Loss of appetite	No.	%	No.	%	No.	%	No.	%	No.	%
+	10	90.9	18	72	8	40	11	68.7	25	64.1
?	1	9.1	7	28	11	55	5	31.3	13	33.3
0	0	0	0	0	1	5	0	0	1	2.5
Nausea										
+	4	36.3	4	16	3	15	5	31.3	6	15.3
?	5	45.4	12	48	7	35	6	37.4	18	46.1
0	2	18.1	9	36	10	50	5	31.3	15	38.4
Vomiting										
+	2	18.1	3	12	3	15	3	18.7	5	12.8
?	5	45.4	12	48	4	20	7	43.7	14	35.9
0	4	36.3	10	40	13	65	6	37.5	20	51.2

For explanation of +, ?, and 0, see Key, Table 2.

It is interesting that in malaria, the drop in serum albumin becomes apparent so rapidly, while in diseases with obvious loss of albumin or decreased protein intake, the reflection in the serum level seems to appear much slower. It seems unlikely that decreased protein intake and depleted protein stores are the entire explanation in the malaria, but perhaps the presence of liver disease in some way inhibits the formation of serum albumin from its precursors. The rapid return of protein values to normal levels after the febrile period also supports this hypothesis.

Nurses were instructed to stand at the patient's bedside until the sodium benzoate was ingested. If it was incompletely taken, if there was any vomiting after its administration, or if there was any question that the total 4 hour specimen of urine was not collected, then such tests were discarded.

Table 3, line C, shows the range and averages of the hippuric acid determinations in the various periods in the 3 groups. The results were similar in all groups. However, although during the febrile period the hipuric acid excretion drops (a

fact not unexpected), the average excretion continues to drop during the period after the termination of fever. This is hardly to be expected in view of the return towards normal of all the other laboratory findings, and requires further comment.

We must assume that the results are factual, even though a number of determinations after the febrile period, and a few during fever, were very low (0.1 to 0.8 gm.), because the techniques were checked both on the ward and in the laboratory.

function of the liver, the use of these drugs may account for the increasing impairment of the detoxification function, as reflected by the hippuric acid excretion test. A comparison of the averages of the hippuric acid determinations with the degree of liver enlargement reveals no correlation of these findings.

(*e*) *Cephalin Cholesterol Flocculation Test.* This determination was done by the method of Hanger, using Wilson Laboratory Antigen. Table 7 shows the readings of the cephalin cholesterol flocculation test

TABLE 7.—CEPHALIN CHOLESTEROL FLOCCULATION REACTIONS (48 HOUR) ON THE 20 PATIENTS IN EACH GROUP BEFORE, DURING, AND AFTER THE FEBRILE PERIOD

	No. cephalin cholesterol flocculation reactions					
	Neg.	+	+	++	+++	++++
Group A—Before	4	2	5	3	1	1
During	2	0	0	0	2	30
After	10	0	1	2	6	15
Group B—Before	7	1	2	4	1	2
During	2	0	0	0	0	31
After	6	2	3	0	9	15
Group C—Before	6	1	3	5	1	1
During	1	0	0	0	2	32
After	7	3	0	3	4	17

TABLE 8.—URINARY UROBILINOGEN DETERMINATIONS ON THE 20 PATIENTS IN EACH GROUP BEFORE, DURING, AND AFTER THE FEBRILE PERIOD

	Group A		Group B		Group C	
	Range	Average	Range	Average	Range	Average
Before	10-40*	28	10-60	26	20-150	35
During	10-300	80	10-800	86	10-250	87
After	10-150	37	20-100	41	20-100	35

* These figures indicate the dilution in which the urobilinogen test was positive, *e. g.*, 40 indicates urobilinogen was positive in 1:40 dilution.

There is a possible explanation, however, as to why the detoxification function may be increasingly impaired after the febrile period. As previously noted, in at least 7 cases the liver enlargement was found to increase after the termination of malaria with atabrine and after the 10 daily injections of mapharsen. Furthermore, the hippuric acid excretion did not drop to the extremely low levels noted above in any of the cases not receiving mapharsen after the febrile course. All of the extremely low results occurred in patients receiving the 10 daily injections. Because both atabrine and mapharsen place an added load on the detoxifying

in 48 hours in the various periods in each group. The readings were entirely similar in all 3 groups. The reaction was 4+ in 48 hours in practically every case of malaria during the febrile period. In only 2 of the 60 cases included in this study did the reaction not reach 4+ in 48 hours. These 2 patients were both in Group A; both had quartan malaria; and both showed only a 3+ reaction in the one reading made in each case during the febrile period. One patient experienced only 8 hours of fever above 102° F., and then developed a spontaneous remission. He was subsequently reinoculated with falciparum malaria in order to complete

the desired therapeutic course. The other experienced only 4 hours of fever above 103.6° F. He had no paroxysms reaching 104° F. but did run a low grade fever for 32 days.

The cephalin cholesterol flocculation tests did not return to normal levels as rapidly as the other laboratory findings. As a matter of fact, at the time of discharge from the hospital, which in most cases was between 3 and 4 weeks after the termination of the malaria, about 50% of the patients still had a flocculation reading of 4+ in 48 hours. This may be an indication that the liver had not returned to its normal state, and such patients should be further followed.

(*f*) *Urinary Urobilinogen Determination.* This test was performed by Wallace and Diamond's modification of Ehrlich's method. Table 8 shows very little difference between the range and averages of the determinations in the 3 groups during the different periods of observation.

(*g*) *Prothrombin Time Determination.* Prothrombin determinations were done by the method of Quick, using thromboplastin made by the Fourth Service Command Laboratory and by the Finney General Hospital Laboratory. The prothrombin time determinations were reported in seconds. Though each group of prothrombin readings were performed with lots of thromboplastin which gave normal readings with normal blood when prepared, there was enough variation between the various lots of thromboplastin to make the determinations more dependent upon the particular lot used and the day the test was done than upon any condition in the patient. For this reason, the only conclusion drawn from these results was that they cannot be reported in seconds if it is desired to compare the determinations made at different times with different lots of thromboplastin on the same or different patients. The results must be reported by some method which allows comparison between various lots of thromboplastin, such as the percentage method outlined by Hurn, Barker and Magath.[2]

3. Histologic Observations. During the course of this study, 2 patients not included in the investigation of liver function died during therapeutic malaria. The pathologic findings in the liver in these cases were as follows:

Autopsy Findings. Case 336. *Death from spontaneous rupture of the spleen.*

Liver. Weight 2100 gm. The surface was dark, reddish tan and finely granular. The edges were rounded. On section the cut surface was dark, reddish tan in color and was more friable than normal. The normal hepatic pattern was present on microscopic examination, but there was moderate thickening of the peripheral connective tissue with infiltration by many round cells and occasional histocytes. The Kupffer cells were swollen and contained reddish brown granular pigment. The hepatic cells showed cloudy swelling and finely granular yellowish green pigment in their cytoplasm. Occasional foci of interstitial hemorrhage were present. The small bile ducts appeared normal. The portal veins contained several mononuclear cells.

Case 445. *Death, primary cause unknown, related to hypocalcemic tetany.*

Liver. Weight 1575 gm. The liver appeared enlarged with some rounding of the lower edge. Color normal but consistency softer than normal. The cut edge everted slightly and the cut surface was homogeneous, reddish brown in color and moderately congested. The lobules were fairly well demarcated. Microscopic examination showed a capsule of normal thickness but generalized enlargement of the hepatic lobules resulting from dilatation of the sinusoids with blood. The sinusoids contained an increased number of mononuclear cells. The histiocytic endothelial cells showed moderate proliferation. The mononuclear cells assumed ameboid shapes, contained a few vacuoles and some granular yellow pigment. The hepatic cells in the liver cord appeared compressed. The central veins displayed congestion and slight subintimal infiltration by mononuclear cells and lymphocytes. The periportal connective tissue showed an increased number of fibroblasts in some areas, resembling a submiliary granuloma. The bile ducts contained a moderate amount of bile.

The increased connective tissues in the periportal spaces and pigment deposition were common findings in the 2 livers. In 1 case, the hepatic cells showed cloudy swelling and occasional foci of interstitial hemorrhage, while in the other, the hepatic cells were compressed, apparently from dilatation of the sinusoids with blood.

These anatomic findings are to be compared to those reported by Kern and Norris.[3] In their 2 cases of naturally acquired malaria, who died of this disease, the findings in the liver at postmortem examination revealed (1) hepatitis, with central and mid-zonal necrosis, and (2) central and mid-zonal atrophy. In 4 cases of acquired malaria, who died of other causes, they found swelling of the parenchymal liver cells and consequent compression of the sinusoids.

From these histologic observations, it can be stated that there is definite evidence of some alteration of structure in the liver during malaria infections. Observations are insufficient to categorize the type of involvement.

Summary. From the results of this study, it appears that the liver is involved in every case of malaria, showing some degree of dysfunction by various tests. The function of detoxification, as measured by the hippuric acid excretion test, was depressed for the longest period, although atabrine and mapharsen administered after malaria therapy may contribute to the continued depression of this function. The involvement of the liver is probably not very severe, since other liver function tests return to normal in a short period after the termination of malaria. The cephalin cholesterol flocculation test, however, returns to normal more slowly than the other tests performed in this study.

This study also indicates that the enlargement of the liver is no indication of the degree of dysfunction. Furthermore, the digestive symptoms experienced during malaria therapy are not dependent upon the liver disease, but apparently largely upon the malaria, probably the fever, itself. Though marked loss of appetite increased in frequency as the blood bilirubin became elevated, the frequency of definite anorexia did not vary significantly with liver enlargement.

Judging by the liver function tests used in this study, there was no striking protection of the liver by the addition of protein (amounting to more than 32 gm. daily) to the diet, high vitamin intake, or crude liver extract injections. However, the addition of 75 gm. of glucose intravenously daily did decrease the incidence of liver enlargement quite significantly.

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EFFECT OF ROOM TEMPERATURE ON SEDIMENTATION RATE OF RED BLOOD CELLS OF MAN*

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This paper is an account of experiments made for the purpose of discovering how the sedimentation rate of red blood cells is changed by room temperature, and if it is possible to make a graph which will give the true sedimentation rate at a given base temperature, for example 20° C.

Table 1.—Sedimentation Rates at 6 Different Temperatures

Unrighted sed. rate, mm:hr.:	55–50	49–45	44–40	39–35	34–30	29–25	24–20	19–15	14–10	9–0
22° C.—N.O.	11	13	14	7	14	21	12	18	17	29
M.	47.0	37.6	31.0	25.3	17.6	14.6	10.7	7.0	4.0	2.4
C.V.	10.0	18.6	14.5	15.0	25.0	24.8	15.9	28.6	35.0	45.8
26° C.—N.O.	9	4	8	5	2	5	7	9	9	16
M.	48.2	38.2	31.2	26.0	18.0	15.0	12.0	7.3	5.0	3.0
C.V.	12.0	13.4	10.9	15.4	23.3	26.7	14.2	23.4	32.0	33.3
30° C.—N.O.	9	13	13	7	14	21	12	18	17	29
M.	49.7	42.0	35.0	29.0	21.6	19.0	15.0	10.5	6.5	3.9
C.V.	8.0	9.5	8.3	9.7	20.8	14.7	9.3	18.1	26.2	41.0
34° C.—N.O.	19	5	8	5	2	6	8	10	9	17
M.	52.0	43.6	37.0	32.4	25.4	21.0	17.5	12.8	8.2	4.9
C.V.	7.7	6.9	6.8	6.2	23.2	17.1	8.0	13.3	15.7	28.6
37° C.—N.O.	11	9	12	6	10	15	10	14	14	23
M.	52.0	43.2	38.0	33.5	27.4	22.0	18.2	13.4	9.0	5.3
C.V.	6.4	8.8	5.3	6.6	9.5	9.1	7.7	11.2	13.3	26.4
41° C.—N.O.	9	9	9	5	5	13	9	13	12	19
M.	53.8	47.0	42.6	37.4	31.5	27.0	22.4	17.0	12.0	6.0
C.V.	6.2	2.1	3.2	2.2	5.4	5.2	4.5	8.8	8.8	28.4

Number of observations (N.O.), mean sedimentation rates (M.), and coefficients of variation (C.V.).

Though we have had knowledge for a long time that room temperature may have an effect on the sedimentation rate of blood, sufficient attention has not generally been given to this possible cause of error. DeCourcy,[1] Fahreus,[2] Gordon and Cohn,[3] Rosenthal,[4] Stats and Wasserman,[5] and Westergren[6] have gone into certain details of this question, but have not made graphs for correction of the error. Graph 2 in this paper makes clear the effect of high room temperature on sedimentation rate. For example, at 37° C., a sedimentation rate of 16 mm. in 1 hour in a young man would be only 8 mm. at 20° C., which is a normal rate for the test of Wintrobe and Landsberg, where the range for healthy men is 0 to 10. In the same way use of a sedimentation rate in which a correction has not been made for the effect of temperature may be the cause of an error in diagnosis of patients, who have been under medical care for a long time, and at different times of year. Comparison of a sedimentation rate measured on a warm day in summer with one measured on a cold day in winter may be the cause of a wrong opinion of the patient's condition. But, if a correction is made to 20° C., which is normal "room temperature," then comparison of observations made at different temperatures is possible.

* This paper is in Basic English. But for a small number of special scientific words, only words in the basic and international science lists are used. The special words are: diagnosis, hematocrit, sedimentation, standard deviation.

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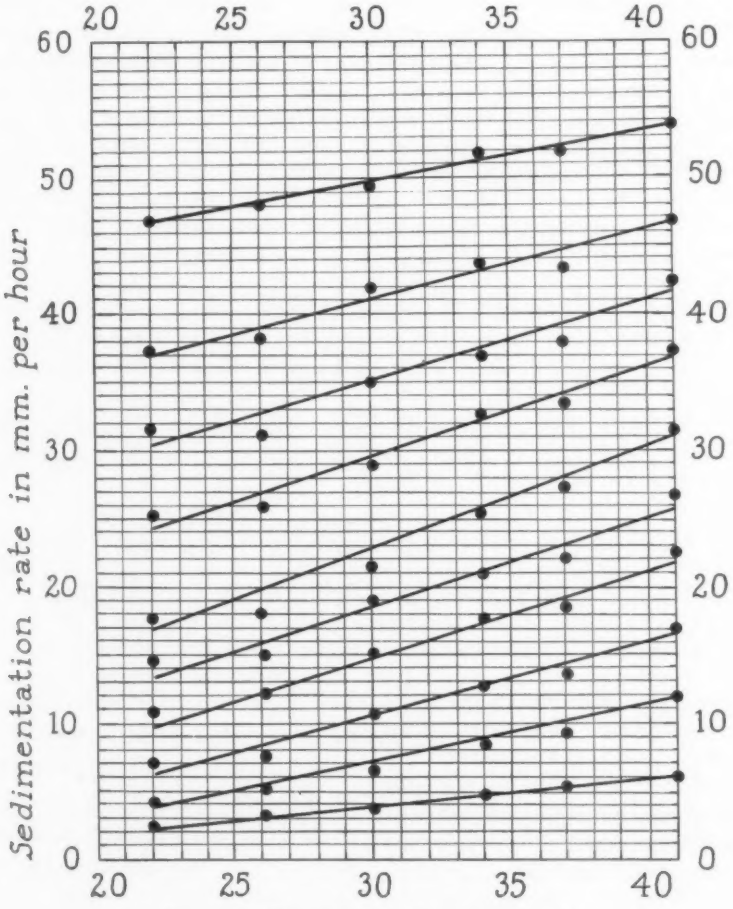
Materials and Ways of doing the Experiments. As much venous blood was taken from 160 patients as was necessary for measuring the red blood cell sedimentation rate by Wintrobe and Landsberg's method[7] at 6 different temperatures. Selection was made of this method because it is widely used and because it gives other observations of value, for example, hematocrit. The patients were all young, white men 18 to 30 years old, who were sick with a number of diseases. No record was made of the diseases, as the cause of the high sedimentation rate was not important for our purposes.

Observations were made at these temperatures: 22° C., or room temperature (this was in fact between 20° and 23° C.); 26°± 0.5° C.; 30°±0.5° C.; 34°±0.5° C.; 37° C (range between 36° and 37° C.); and 41°± 0.5° C.

Blood was put in glass vessels at body temperature, and observations were made at the temperatures given above. Correction was made for hematocrit errors at room temperature at the end of every test in the normal way.



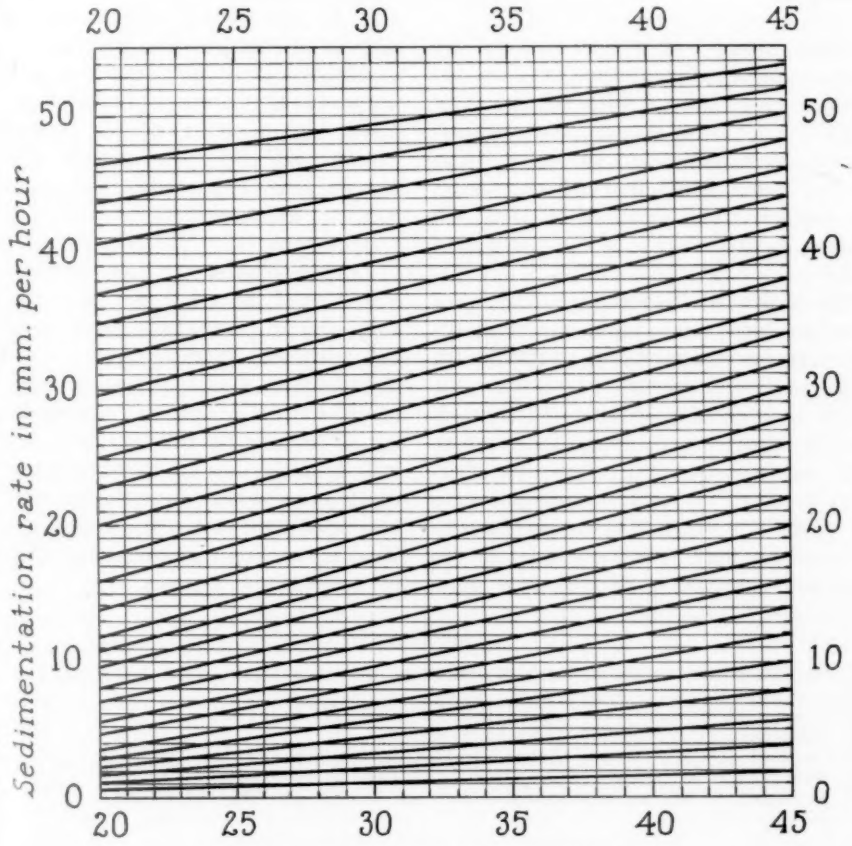
GRAPH 1.—Temperature in degrees centigrade.

Observations. A small number of tests made it clear that temperatures between 15° and 22° C. do not have an important effect on the sedimentation rate, and for this reason no other observations were made at temperatures lower than 22° C.

One hundred and sixty sedimentation rates

were measured. At 22° C. the values were in the range 0 to 60 mm. in 1 hour, whereas at higher temperatures the values were increased. In general, it may be said that the higher the room temperature the quicker the sedimentation rate, and that this relaship may be put in the form of a straight line. To make a statistical analysis of the observations, all readings at 41° C. were put in groups of 5 mm., and means for each group were worked out with standard deviations and coefficients of variation. Like values were used for each of the other 5 temperatures (Table 1). Readings at 41° C. were taken as a starting place or base line for making graphs, because we had the desire to see what the true sedimentation rate of blood would be at 20° C. Graphs were made by putting the means of the sedimentation rates for each temperature on graph paper and making a straight line through the points. Other lines were then put in place on the base line at distances of 2 mm. (Graph 2).

The process of making Graph 2 must be written about at greater length. Means of the experimentally observed sedimentation rates are recorded in Graph 1, from which we see that it is possible to make a straight line through the points in such a way that there is good distribution of points over and under the line. In fact, the distance of these points from the lines is no greater than the coefficient of variation of the means. But it is seen that all lines are not parallel. Probably the reason for this is that, as the sedimentation rate is increased, red blood cells are pushed tighter and tighter together, and the lines get more and more nearly parallel with the base. In other



GRAPH 2.—Temperature in degrees centigrade.

words, in a number of conditions complete or nearly complete sedimentation of cells may take place so quickly that temperature will have but a small effect. Though these facts are so, parallel lines may be placed between those made from the experimental observations without causing a greater error than is present in the test itself. In this way a graph can be made for correction of errors caused by changes in room temperature.

From Table 1 it is seen that at low temperatures sedimentation rates in the normal range have a high coefficient of variation. This is because the measurements are in millimeters so that a difference of 1 mm. which makes little change, for example, in a mean of 30 mm. will make a great change in a mean of 4 mm. or less.

Use of Graph. To make use of the temperature correction graph, the sedimentation rate is measured in the normal way. If the room temperature is high the value at 20° C. is given by looking on the graph for the point where room temperature and sedimentation rate come together. Now go down the nearest sloping line as far as 20° C. where the true sedimentation rate is seen. For example, if the sedimentation rate is 22 mm. in 1 hour at 38° C., the sedimentation rate at 20° C. will be 12 mm. After making the necessary temperature correction, the hematocrit error may be overcome by using the graph made by Wintrobe and Landsberg.[7]

Chief Points of Paper. 1. Observation was made of the effect of high room temperature on sedimentation rate of red blood cells as measured by Wintrobe and Landsberg's method.

2. A high room temperature, such as may be present in warm weather or in an overheated room, may be the cause of an important increase in sedimentation rate, which may make for an error in diagnosis.

3. A graph for correcting the effect of temperature was made from measurements of sedimentation rate at 6 different temperatures (22°, 26°, 30°, 34°, 37° and 41° C.) in 160 sick men 18 to 30 years old.

4. Use of the graph will give the true values at a given base temperature of sedimentation rates which have been measured at a higher temperature (20° to 45° C.). The suggestion is made that 20° C. be used for this base temperature.

5. If corrections are made for the errors which are caused by temperature and hematocrit, then comparison can be made of sedimentation rates measured at different room temperatures.

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APLASTIC ANEMIA IN SOLDIERS TREATED WITH ATABRINE (QUINACRINE)

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Drug suppressive treatment of malaria insured the success of our military operations in the South and Southwest Pacific Areas where many of the islands are among the most highly malarious regions in the world. It also made possible maintenance of our armed forces in certain areas of the Asiatic (China-Burma-India) Theater. Bitter experiences early in the war proved that malaria could neutralize our combat forces far more rapidly and effectively than enemy fire. Other means of malaria control, apart from the use of protective clothing and mosquito repellents, were not feasible until the units were firmly established.

Although drugs naturally did not prevent infection, the dose of atabrine routinely employed for suppressive treatment (0.6 to 0.7 gm. per week in daily doses of 0.1 gm. each) seemed to cure falciparum malaria unless continued reinfection occurred. The symptoms of vivax malaria were suppressed in most cases as long as therapy was continued. Consequently suppressive treatment was carried on until infected individuals were in a situation where active treatment was possible.

Atabrine proved superior to quinine for suppressive treatment, being more effective and better tolerated by the troops. It was generally regarded as harmless and, for the most part, with good reason. Intestinal disturbances occasionally followed the first few doses of atabrine but usually disappeared, even though treatment was not interrupted. Sometimes quinine had to be substituted when intolerance to atabrine became evident.

The possibility that atabrine in suppressive dosage might produce harmful effects was entertained by some observers. In an effort to determine whether this was valid, the Army Institute of Pathology instructed all laboratory officers to include any existing records of atabrine therapy in autopsy protocols no matter what the cause of death. A microscopic examination of tissues and a review of postmortem findings were carried out by staff members of the Institute. For some time nothing of significance was observed, but later it became apparent that aplastic anemia was the cause of death in a disproportionately large number of cases represented by autopsy material sent from the South and Southwest Pacific Areas, where a rigid antimalarial régime was in force. This trend was continuous. In addition, material from sternal bone marrow biopsies of a few non-fatal cases was received from the same theaters.

This report is based on a study of 57 cases of aplastic anemia with symptoms first becoming apparent when the patients were in the South or Southwest Pacific Areas, or the Asiatic (China-Burma-India) Theater. The incidence of the condition in these regions is contrasted with that in the Army elsewhere. For the sake of brevity the troops stationed in the continental and territorial United States, and all foreign theaters exclusive of the areas specified above will be designated as *Group I*, and those in the special areas as *Group II*.

Incidence. The polygons in the upper section of Chart 1 represent the actual number of cases of aplastic anemia which occurred in Group I according to the month during which the initial symptoms were noted by the patients. The corresponding curve in the chart shows the

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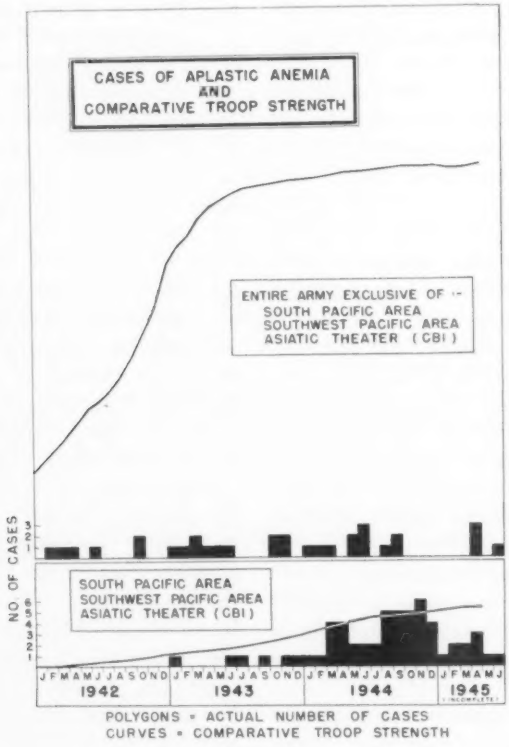
total troop strength of Group I and is drawn to scale for comparison with the troop strength curve of Group II below. The polygons in the lower section tally the cases of aplastic anemia from Group II by month of onset.

It is apparent that aplastic anemia in Group I does not increase at a rate out of proportion to rising Army personnel, whereas the incidence in Group II mounts enormously. This disparity becomes all apparent decline of incidence in Group II during the first half of 1945 is of dubious significance, due to incomplete figures. The corresponding value for Group I is apt to be more nearly complete, however, as virtually all laboratories in the European Theater have been closed and their cases already collected at the Institute; furthermore, material from hospitals in the United States is generally sent in


CASES OF APLASTIC ANEMIA
AND
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ENTIRE ARMY EXCLUSIVE OF -
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POLYGONS = ACTUAL NUMBER OF CASES
CURVES = COMPARATIVE TROOP STRENGTH

CHART 1.

the more striking when the incidence is computed semi-annually as cases per 100,000 troops (Chart 2). Group I has a rate ranging from 0.04 to 0.18 cases per 100,000, the larger figure probably being fortuitously high, as it occurred when the Army was relatively small. It has not been reached again during any subsequent $\frac{1}{2}$ year period. After an initial lag, Group II displayed a precipitous rise from 0.66 to 2.84 cases per 100,000. The rather promptly. No data are available for the last half of 1945.

ETIOLOGY. The years 1943 and 1944 have been selected for a comparative study of the causes of aplastic anemia in both groups, since this was the period in which returns are complete and in which Group II is significantly represented. The results are shown in Chart 3.

In *Group I* aplastic anemia twice followed arsenical treatment of syphilis;

3 times, irradiation of malignant tumors; 4 times, administration of sulfonamides; in 11 cases there was no demonstrable cause. One patient from the North African Theater not included in the tabulation was said to have taken atabrine during the previous 18 months, but there was no information regarding the amount or regularity of the dose.

All had received suppressive doses of atabrine over periods varying from 1 to 34 months, the majority between 4 and 14 months. The 34 month case was unique, however, and the patient apparently had not adhered to the prescribed régime, as his record showed repeated "break-through" of tertian malaria which required active treatment. There was specific mention of overdosage prior to the onset of illness in 6 cases. Four men had increased the dose to 0.2 gm. daily,

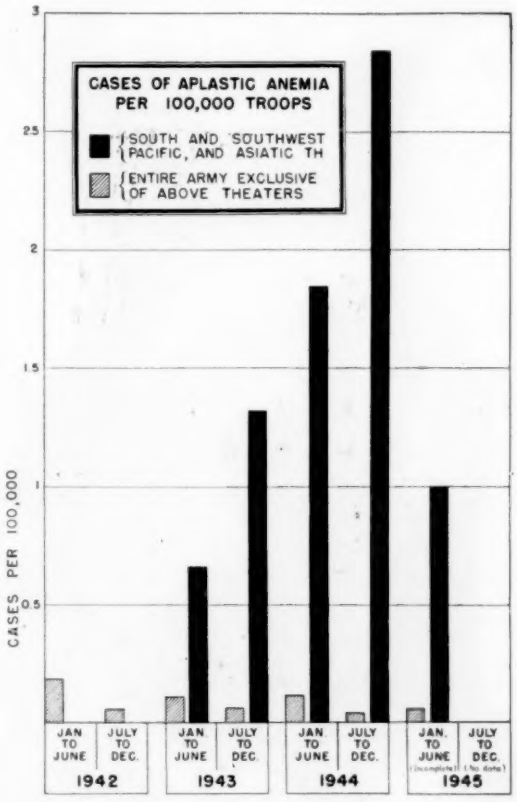

CHART 2.

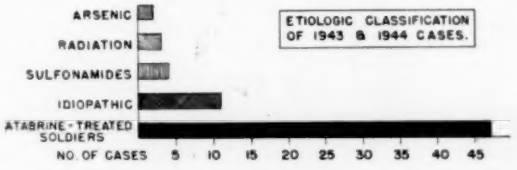

CHART 3.

The 47 cases occurring in *Group II* during 1943 and 1944 are indicated in Chart 3 as "atabrine-treated soldiers."

1 for 3 weeks, another for 6 months, a third for 8 months, and the last for an unspecified time. Another soldier took between 20 and 30 tablets during the 4 days preceding onset of symptoms; still another was said to have ingested "massive doses" for 3 weeks before. Other instances of overdosage probably occurred, but were either not known or not recorded. For example, men on patrol or detached service were given supplies of atabrine adequate for estimated time away from their units and, although instructions were given, administration was unsupervised.

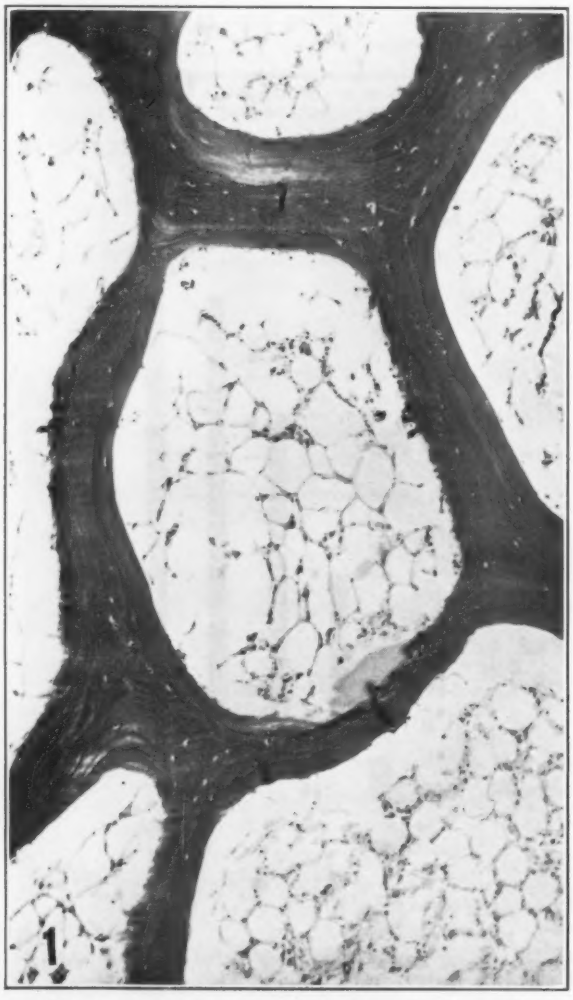

FIG. 1.—Typical sternal bone marrow of a patient dying from aplastic anemia of 2½ months' duration. Marrow spaces are almost completely filled with fat, the cells sprinkled through the interstices being residual erythrocyte and granulocyte progenitors admixed with lymphocytes, plasmocytes and histiocytes. (Reduced from × 125.)

Seven of the group treated with atabrine had also taken sulfathiazole or sulfadiazine. In 2 instances it was not possible to exclude this as a precipitating factor, but in the others it was obvious

that the drugs were administered only after the onset of the anemia and in 2 cases aggravated the purpuric manifestations. Atabrine dermatitis complex, the term recently proposed to designate so-called New Guinea lichen planus, preceded aplastic anemia in 20 cases; in these topical applications of various sorts were used and light Roentgen ray treatment was given in 1, but these were not regarded as significant causal factors. A few patients had taken aspirin for headache which was frequently a feature of the prodromal period. The remainder had

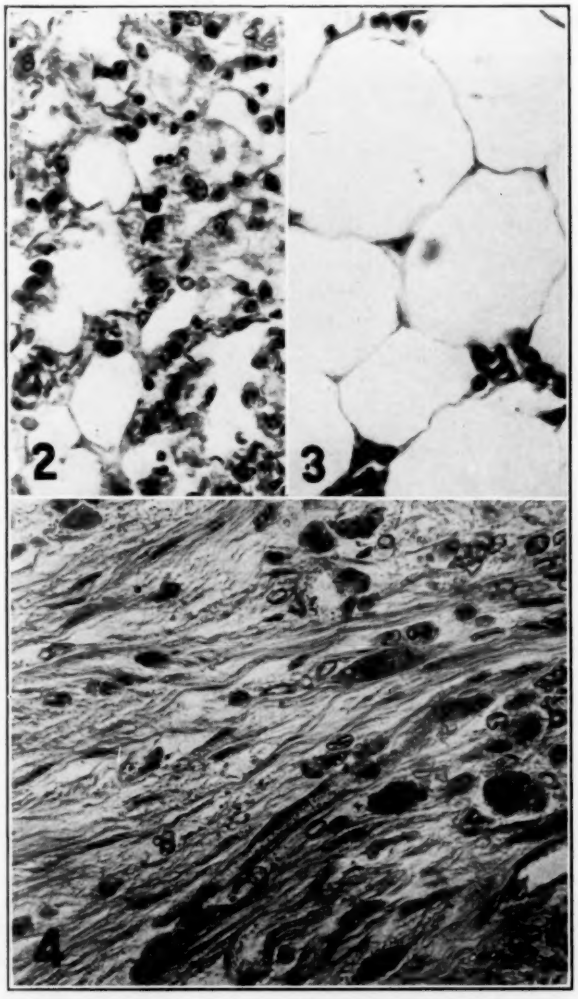

FIG. 2.—The sternal marrow from an acute case (death in 10 days) is largely depleted and shows early fat replacement. The fat cells are small in contrast with those in Figure 3 and some are rather ragged. The remaining cells are mostly erythroblasts, normoblasts, and histiocytes. There is also hemorrhage in the interstices which in some cases is extensive. (Reduced from × 550.)

FIG. 3.—An orderly and nearly complete fat replacement is noted in the marrow of a patient who survived 5 months. The small clusters of nucleated elements are chiefly normoblasts. (Reduced from × 550.)

FIG. 4.—A sternal biopsy performed on this patient early in the course of the disease disclosed a fatty marrow. At autopsy 10 months later marrow spaces of all bones examined showed this extensive secondary myelofibrosis. Hemosiderin-laden histiocytes are conspicuous in the fibrillar mat. (Reduced from × 550.)

received no drugs, nor could a history of exposure to other etiologic agents be elicited.

It would appear that atabrine is responsible for the *relatively* high incidence of aplastic anemia in troops given extended suppressive therapy.

The following descriptions are based entirely on material from Group II in which atabrine is thought to have been the significant etiologic agent:

PATHOLOGY. The *bone marrow* in all cases was badly depleted of normal hematopoietic elements, often almost totally so (Fig. 1), and there was no evidence of extramedullary hematopoiesis. Usually the residual cells of the marrow belonged to the erythropoietic series and were in

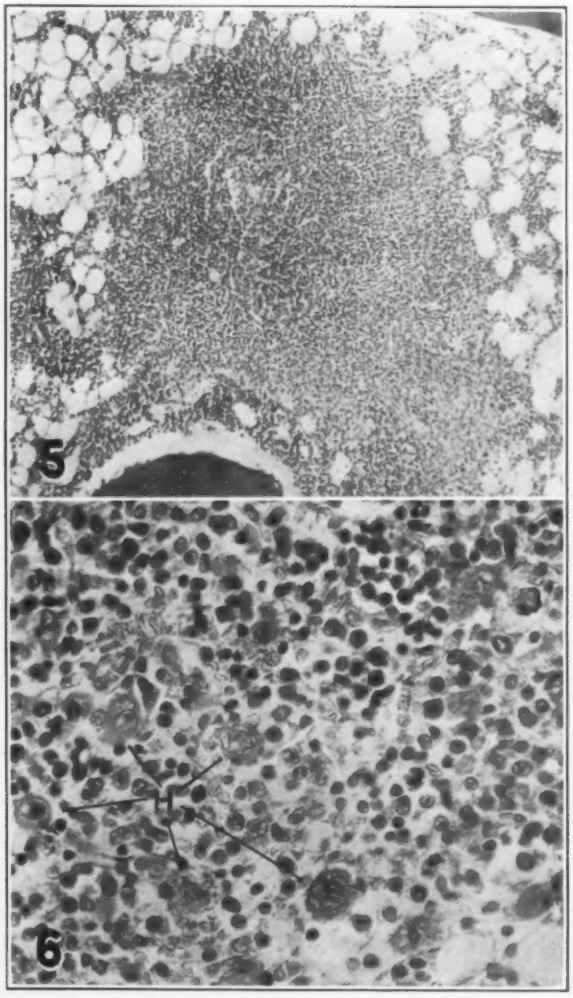

FIG. 5.—A large folliculoid aggregate of lymphocytes in an otherwise poorly cellular marrow. This is a frequent finding, although the lymphoid nodules are not usually as large as the one illustrated. (Reduced from × 125.)

FIG. 6.—A more diffuse influx of lymphocytes, plasmocytes, and histiocytes (*H*), the histiocytes displaying marked erythrophagocytosis. This sort of infiltrate may sometimes occupy fairly large areas of the sections, but other portions disclose the basic aplastic state of the marrow. (Reduced from × 800.)

the late erythroblastic and normoblastic stages. The few granulocytes were generally stab or segmented forms, although occasionally small foci of myelocytes or even younger elements were encountered. Megakaryocytes were either absent or very sparsely distributed and when present often showed evidence of degeneration. The marrow spaces were replaced by fat, raggedly in the acute cases, neatly in those of longer standing (Figs. 2 and 3). One man who lived for 10 months and received 65 blood transfusions had extensive secondary fibrosis of the marrow cavity of all bones examined (Fig. 4). There was an influx of lymphocytes and plasmocytes (Figs. 5 to 8), slight in some cases and conspicuous in others, the lym-

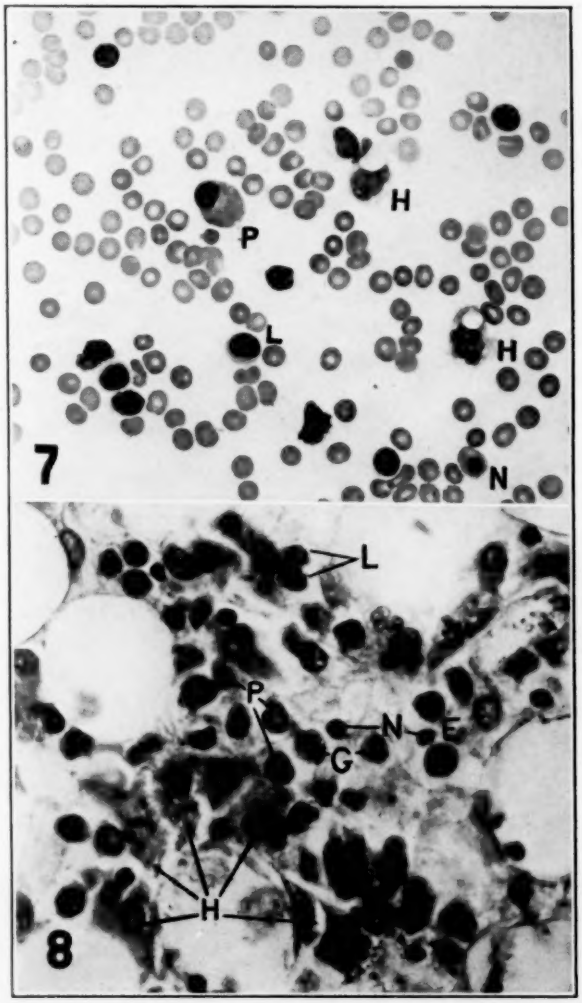

FIG. 7.—A typical sternal aspirate from a patient ill 6 months showing almost complete depletion of the marrow. Most of the cells are lymphocytes (*L*), with a interspersion of histiocytes (*H*) and a plasmocyte (*P*). Hematopoietic elements are represented by the single normoblast (*N*). The histiocytes each contain a faded erythrocyte. (Reduced from × 600.)

FIG. 8.—Detail of one of the residual cell clusters in the marrow of a patient who survived for 2 months. Sample cells are indicated as: *L*, lymphocytes; *P*, plasmocytes; *H*, histiocytes; *G*, granulocytes (a myelocyte and a metamyelocyte); *E*, erythroblasts (above and below the letter); *N*, normoblasts. (Reduced from × 800.)

phocytes frequently aggregating to form pseudofollicles (Fig. 5). Histiocytic proliferation was usually very pronounced in patients who survived for several months and received many transfusions; most of the cells contained hemosiderin and erythrocyte fragments. Sometimes this combination of lymphocytes, plasmocytes, and histiocytes attained such proportions that at first glance the fundamental hypoplastic state was not apparent (Fig. 6). For example, in 1 such instance the appearance of the bone marrow was interpreted as evidence of aleukemic lymphatic leukemia by the laboratory officer who submitted the case.

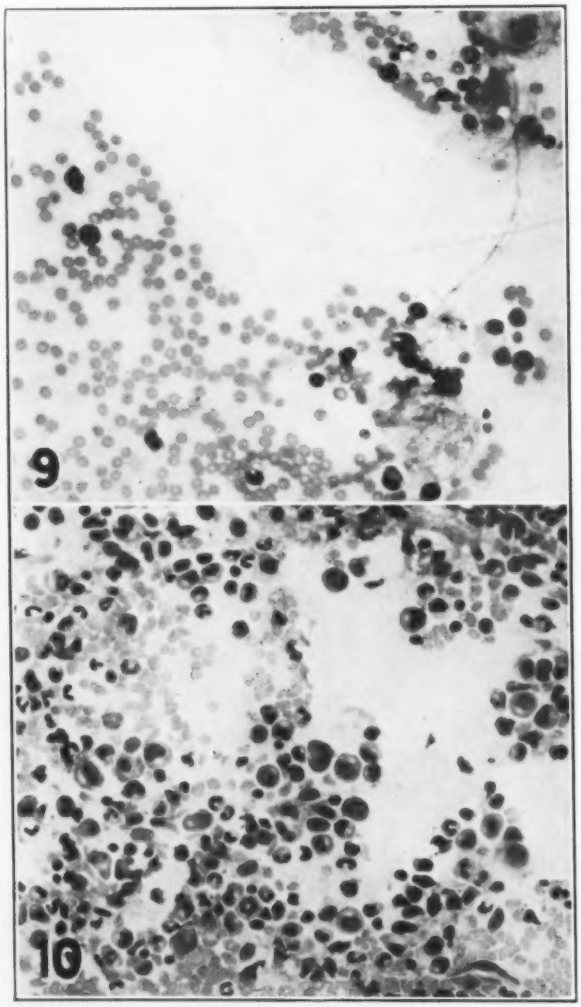

FIG. 9. Sternal aspiration disclosing a markedly hypoplastic marrow on the 17th day of illness. The 2 degenerated megakaryocytes shown in the upper right were the only ones observed in the smears. The aspirate contained considerable quantities of fat and serous fluid which blurred the preparations to some degree. (Reduced from × 300.)

FIG. 10.—Aspirated marrow from the same case on the 72nd day showing a strong regenerative activity, particularly of the granulocyte series. The clinical state of the patient was correspondingly better and there was promise of complete recovery. (Reduced from × 300.)

Bone marrow findings during life are described in later paragraphs dealing with sternal biopsies.

Apart from the marrow changes, *hemorrhage*, involving the skin, mucous and serous membranes, and viscera to varying degrees, was the conspicuous feature in virtually the entire series. Cerebral hemorrhage was the immediate cause of death in 10 cases (Fig. 13), occlusive laryngeal hemorrhage in 1 (Fig. 11), and massive gastro-intestinal hemorrhage in 2 others (Fig. 12). Submucosal hemorrhage in the colon was occasionally followed by surface sloughs simulating those in acute ulcerative colitis. Bleeding along the urinary tract was an almost invariable finding (Fig. 14).

Infection was not so prominent as one would expect in the presence of constant and profound neutropenia, probably because penicillin had been used freely in most cases. There was evidence of bacteremia in only 6, bronchopneumonia in 8, and urinary tract infection in 1.

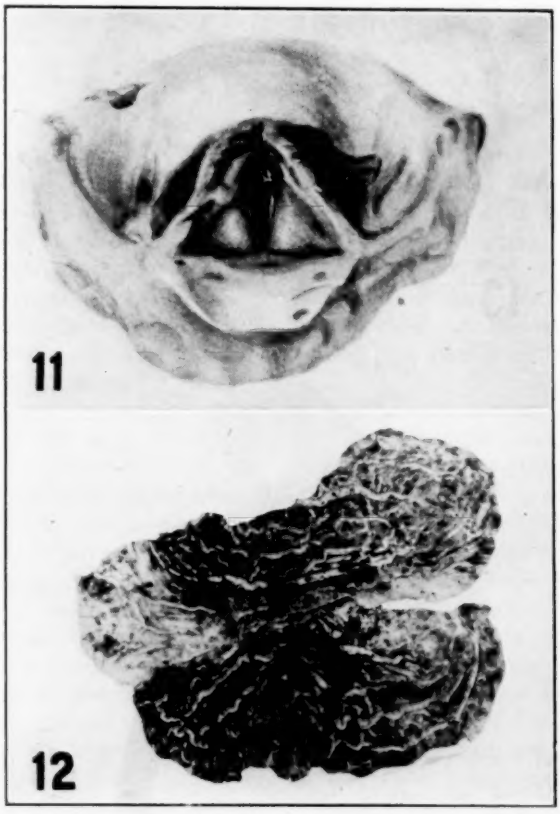

FIG. 11.—The larynx of a patient who died of asphyxia, the airway having been completely occluded. Apposition of the true vocal cords resulted from extensive submucosal hemorrhage.

FIG. 12.—Punctate and diffuse bleeding in the stomach of a patient who died soon after a series of gastro-intestinal hemorrhages. The colon presented a similar appearance with the addition of superficial sloughs.

Liver lesions were observed in 10 cases (Chart 4), in 5 being indistinguishable from epidemic hepatitis as described by Lucké.[1] Active periportal hepatitis was seen in 3 cases, focal intralobular hepatitis in 1, and central phlebitis in another. The liver lesions were probably incidental,

particularly those resembling epidemic hepatitis which was widespread in the areas from which the cases were sent.

The *atabrine dermatitis complex* which preceded the anemia in 25 cases (Chart 4) is described in detail in a separate report

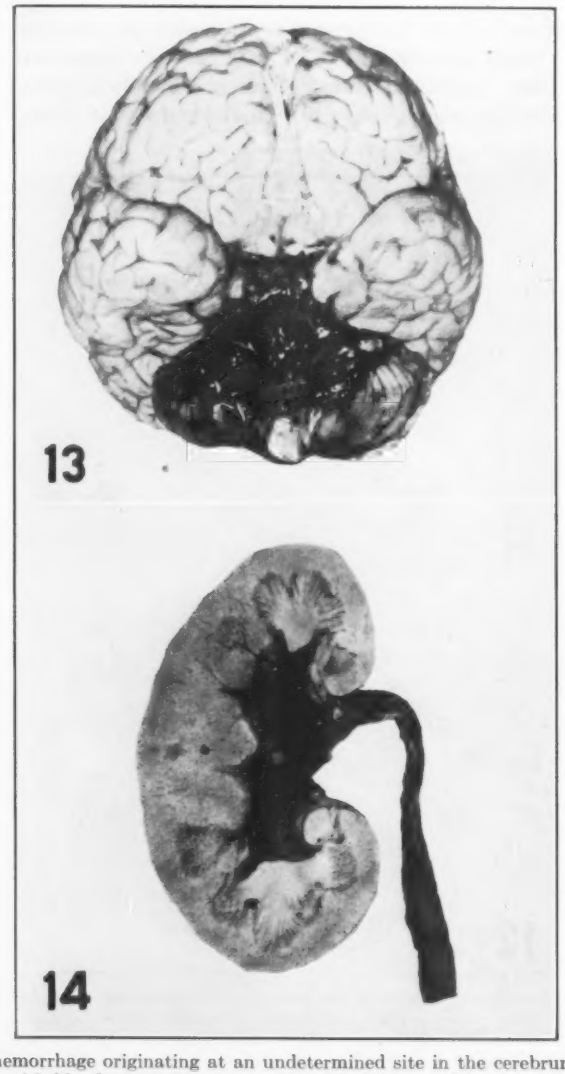

FIG. 13.—Basal hemorrhage originating at an undetermined site in the cerebrum, filling the lateral and third ventricles with blood, rupturing the septum pellucidum, and breaking through into the subarachnoid space.

FIG. 14.—Hemorrhage in the renal pelvis extending along the ureter resulted in extensive hematuria, a frequent finding in the series.

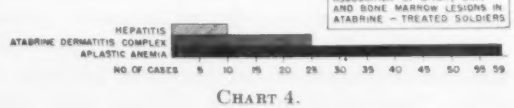

CHART 4.

from this Institute by Mullins and Allen, so that these pathologic changes will not be discussed here.

Tissue atabrine values, available in only 1 case, were reported as follows:

Site	Atabrine (mg./kilo)	Atabrine degradation products (mg./kilo)
Liver	16.8	1.7
Spleen	16.2	0
Kidney	6.6	0
Skin, normal, chest	6.6	0
Skin, normal, thigh	12.9	0
Skin, Lesion A	5.9	0
Skin, Lesion B	7.1	0

Bone marrow: 3.5 gamma atabrine extracted from 20 gm. of rib; no degradation products. (This corresponds to 0.175 mg./kilo and is probably not a valid figure.)

This patient took 0.2 gm. of atabrine daily for 10 days beginning May 30, 1944, followed by 0.1 gm. daily until Feb. 28, 1945, 24 days prior to death.

Clinical Manifestations. The most common complaint of the *prodromal period* was weakness, often associated with headache, vertigo and dyspnea. A hemorrhagic tendency was frequently noted early, evidenced by bleeding gums after brushing teeth, nosebleed, "bruising easily," or the spontaneous appearance of ecchymoses in the skin. Dimness of vision noted first by 2 of the patients was found to be due to intraocular hemorrhage. In the majority of cases the onset was gradual, several weeks to a month or even more elapsing before the men reported to sick call. The prodrome was of brief duration in 6 cases and the onset precipitous in 3 in which an acute febrile state was the first indication of illness.

The early manifestations of aplastic anemia were often unnoticed in the group of 25 patients who were being treated for skin lesions until individuals among them were observed to be rather pallid and a blood count disclosed a reduction in the formed elements of the blood. In no instance did the anemia precede the dermatitis.

The *course* was almost invariably marked by fever and hemorrhagic phenomena, the location and extent of the hemorrhages accounting for differences in symptoms. For example, hemiplegia, convulsions, and coma were noted in patients with intracranial bleeding, or hematuria and sometimes flank pain with urinary tract hemorrhage. Four patients were still living at the time of the last report, 1 having survived 2 months, a second 4 months, and the other two 7 months.

Hematologic Findings. Rather complete series of blood counts were submitted in most cases, and the diagnosis of aplastic anemia was confirmed by sternal biopsy in 25.

In 4 cases blood counts were performed very soon after the onset and showed that the red cell level was fairly well maintained (4,000,000 to 5,000,000 per c.mm.), whereas the leukocyte and platelet counts had fallen sharply, the lowest being 1500 and 40,000 per c.mm. respectively. This is to be expected when one recalls the relatively long life of the mature erythrocytes (average 60 days as opposed to 3 to 5 days for neutrophils and platelets), and that most of the residual cells found in the bone marrow at autopsy are their progenitors.

The other patients were already anemic when first examined and initial erythrocyte counts as low as 600,000 per c.mm. were recorded, although the average range was 1,500,000 to 3,000,000 per c.mm. The anemia was generally of the normocytic, normochromic type; in some cases it seemed to be hyperchromic, but this is questionable since it was reported only where anemia was severe, *i. e.*, in the range where hemoglobin readings are apt to be very inaccurate. Furthermore, no hematocrit values were available in these cases to check for macrocytosis. Nucleated red

cells almost never appeared in the peripheral blood. Reticulocyte counts over 1% were rarely recorded and they were frequently reported as either less than 0.1% or "none found." Anisocytosis and poikilocytosis were seldom marked except in the few cases showing evidence of regeneration. It was possible with frequently repeated blood transfusions to restore the erythrocyte and hemoglobin levels to normal or nearly so in 16 cases and to effect an improvement in nearly all. A few patients became progressively more anemic despite transfusions and the majority did not maintain their bettered status.

The initial leukocyte count was seldom over 3000 per c.mm. and usually fell between 1000 and 3000, being normal (7200) in only 1 case. Values below 1000 per c.mm. were noted in 5 instances. The counts fluctuated somewhat, and occasionally rose following transfusions, but the general trend was toward lower levels as the disease progressed, with little tendency to increase significantly even during clinical remissions. The leukopenia was due primarily to failure of the bone marrow to produce granulocytes, but when the total count was greatly reduced the relative lymphocytosis was actually a lymphopenia. This was also the case with respect to the monocytes. Absolute agranulocytosis was frequently recorded, but a general scan of the blood films usually disclosed a few neutrophils, most of them segmented. Immature granulocytes were occasionally seen early in the course, but rarely thereafter.

Blood platelet counts were significantly low in all but 4 of the 49 cases in which they were done. In 3 of these exceptions only 1 platelet count was made and that early in the course of the disease; in the fourth 2 counts were recorded as 263,000 and 154,000 per c.mm. Autopsy disclosed extensive visceral hemorrhages in every one of these cases, however, and the marrows were devoid of megakaryocytes, indicating that subsequent platelet levels must have been much reduced. In general the counts ranged between zero and 100,000 per c.mm., most below 50,000 at some time during the disease.

The coagulation time was little altered, but clot retraction was slow or absent. Bleeding time was sometimes normal even though the tourniquet test was positive; in other cases the bleeding time was prolonged, the lancet prick oozing for several days in 1 instance.

Aspirates of the *sternal bone marrow* were studied in 23 cases and fragments of the bone in 2, the latter showing the characteristics already described. Fat was conspicuous in many of the slides prepared from the aspirated material and the nucleated cell count ranged between 5000 and 10,000 per c.mm. in the few instances in which it was recorded. As one would anticipate, the differential bone marrow cell counts varied considerably with the technique employed and the degree of dilution with peripheral blood. Sample counts are shown (in percentages) in the following tabulation. Cases A and B terminated fatally. In Case C there was promise of recovery, both clinically and from the change in character of the successive marrow biopsies; C (Fig. 9) was taken after 17 days of illness, C′ (Fig. 10) after 72 days.

The count in Case A and the first count in Case C would imply either nearly complete aplasia of the marrow or extensive dilution with peripheral blood. The presence of considerable fat in the smears, however, in addition to plasmocytes and hemosiderin-laden histiocytes, signifies that they are representative specimens of the marrow. The second count in Case C shows spontaneous regeneration after the patient had been tided over the aplastic phase with 22 blood transfusions. The marrow aspiration in Case B was performed during the early retrogressive period of the disease and the count reflects some residual hematopoietic activity; the autopsy 1 month later disclosed virtually complete aplasia apart from a few tiny foci of normoblasts. These few but representative examples indicate that biopsy of

the sternal bone marrow is usually of diagnostic and prognostic significance.

Discussion. During a period for which records are reasonably complete (1943 and 1944), nearly $2\frac{1}{2}$ times as many cases of aplastic anemia occurred among troops stationed in the South and Southwest Pacific Areas and the Asiatic Theater combined as in the entire Army elsewhere, even though the troop strength in these regions varied from one-twenty-fifth to one-seventh that of the larger group (Chart 1). Expressed as cases per 100,000, this disproportionate incidence is even more striking and it shows a rapid and steadily progressive increase with each succeeding $\frac{1}{2}$ year period (Chart 2) up to the first half of 1945, the returns from which are incomplete. In contrast, the incidence of aplastic anemia in the Army exclusive of these particular areas remains remarkably constant.

In the malarious areas of the Pacific and Asiatic Theaters 0.1 gm. of atabrine was given 6 or 7 times a week continuously and for at least 4 weeks after redeployment to non-infected zones. Directives called for the drug to be administered by roster to both officers and men, with a competent non-commissioned officer to witness the actual swallowing of the tablets. Although there undoubtedly was a certain amount of laxity in the earlier months, the value of suppressive treatment became so apparent that failure to comply became a matter for disciplinary action. This regime differs radically from that employed in the Mediterranean Theater where the usual dose was 0.3 gm. per week and the supervision sometimes desultory; furthermore, suppressive medication was omitted entirely during the winter.

Thus, apart from geographic location, the single common factor unique to troops in the tropical Pacific and Asiatic Theaters was the protracted use of atabrine. Were it possible to eliminate from consideration the men stationed in non-malarious or sanitated zones within these theaters the incidence of aplastic anemia mong those actually taking atabrine would be still higher. Even if the few patients who also receive sulfonamides were excluded, the incidence curve would remain significantly high. It has been suggested that the surreptitious use of sulfonamides as a venereal prophylactic might have been responsible for some cases, but this practice was unquestionably more prevalent in the European and Mediterranean Theaters than in the Pacific Islands.

It has been fairly well established that the so-called New Guinea lichen planus is caused by atabrine and the condition has recently been designated "atabrine dermatitis complex." It may be of some significance that in 25 of the 57 cases these skin lesions preceded the development of the anemia. Therapeutic agents directed toward the dermatitis were not recognized as likely to produce aplastic anemia except

	Case A	Case B	Case C	Case C
Myeloblasts	0	2	0	3.5
Neutrophil:				
Myelocytes A	0	4.5	0	15
Myelocytes B	0	7.5	0	10
Myelocytes C	0	5	2.5	7
Stab forms	0	5	1	12
Segmented forms	4	4	2	8
Eosinophils	1	0	0	0
Proerythroblasts	0	9	0	6
Erythroblasts	1	18.5	1	10
Normoblasts	3	7.5	1	12
Megakaryocytes	1	0	0.5	0.5
Lymphocytes	81	30.5	80	11
Plasmocytes	5	3	4	2
Monocytes	1	1	3	1
Histiocytes	3	2.5	5	2

the single case in which Roentgen ray was used, but here the dosage employed was too small to be regarded as dangerous.

For these reasons atabrine is believed to be the cause of most cases of aplastic anemia occurring among troops receiving drug suppressive treatment continuously for months. The rapid rise in rate per 100,000 over 4 successive ½ year periods is probably a reflection of the length of treatment, perhaps influenced also by an increasingly rigid discipline regarding administration of the drug. The individuals may have slowly acquired hypersensitivity, the antigen possibly being formed through the interaction of the drug or one of its radicles and a protein. The rôle of overdosage cannot be satisfactorily evaluated.

Conclusions. 1. Atabrine administered in suppressive doses over a period of months may cause aplastic anemia.

2. The incidence of aplastic anemia among troops treated continuously with atabrine is far higher than among other soldiers.

3. The actual number of cases of aplastic anemia attributed to the use of atabrine is infinitesimal as contrasted with anticipated morbidity and mortality from malaria had drug suppressive therapy not been employed.

4. The increased rate of aplastic anemia among troops given atabrine in suppressive doses does not constitute a contraindication to the use of the drug.

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PROGRESS
OF
MEDICAL SCIENCE

OPHTHALMOLOGY

UNDER THE CHARGE OF
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TEMPORAL ARTERITIS AND LOSS OF VISION

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When Horton, Magath and Brown[7] first described the syndrome of temporal arteritis in 1932, they regarded it as an essentially localized or isolated arterial lesion, though recognizing that the systemic manifestations were disproportionate to the local arterial lesions. In a further report in 1937, Horton and Magath[8] emphasized that the subjective symptoms, the clinical findings and the histologic changes in excised portions of the temporal arteries were so typical and so essentially identical in all cases as to constitute a new, or at least previously undescribed clinical entity. Cases described subsequently by other authors confirmed the actuality of this entity but showed also that the involvement of arteries might be more generalized than was suspected originally. In fact, in 1943, Horton[10] stated: "We always have suspected that the arteritis in this condition was more extensive than could be detected on careful physical examination. Recent observations confirm this impression." A recent editorial[5] recognizes temporal arteritis as a new disease entity. It calls attention to the fact that the studies of Gilmour,[6] of Sproul,[15] of Chasnoff and Vorzimer,[2] and of Cooke and his associates[3] have demonstrated that "arteritis of the temporal vessels is probably a local manifestation of a systemic arterial disease which cannot be regarded as nonfatal."

Perhaps the first clinical evidence that the arterial disease in this syndrome was not confined to the temporal vessels was furnished in 1938 by the occurrence of bilateral blindness due to closure of the central arteries of the retinas in a case of temporal arteritis observed by Jennings and Comb.[9] Horton and Magath[8] stated in 1937 that evidence of phlebitis of one of the retinal veins with hemorrhages and exudates had been observed in 2 of their cases. It seemed difficult to connect this in any direct way with the arteritis of the temporal vessels. However, the report of Jennings and Comb[9] was followed by reports of similar cases by Dick and Freeman,[4] by Scott and Maxwell[13] and by Johnson, Harley and Horton.[10] Thus it became evident that loss of vision was not a chance accompaniment but a definite part of the syndrome of temporal arter-

itis. It appears that involvement of vision is to be expected in about one-third of patients who have arteritis of the temporal vessels. Johnson, Harley and Horton[10] suggested the possibility of a direct extension of the lesion from the temporal arteries into the arteries of the optic nerves. They stated: "The anterior deep temporal arteries have an anastomosis with the lacrimal branch of the ophthalmic artery. The ophthalmic artery gives off the central retinal artery, which sends an anterior and posterior branch to the optic nerve. It would be conceivable that the inflammatory process in this case had traveled in this direction." In view of the demonstration of lesions in arteries in various parts of the body in this syndrome, demonstration of such direct extension of the process would not seem to be necessary to explain the involvement of the central artery of the retina.

As stated by Cooke and his associates,[3] "There exists in elderly people a widespread arterial disease, not uncommon, but rarely recognized, in which characteristic arterial and striking local signs occur, and in which inflammatory and degenerative changes in the walls of the affected arteries produce a characteristic histologic picture." These authors state that 37 cases have been reported, in 18 of which there was some visual disorder. The patients varied in age between 55 and 80; 25 were among women and 12 were men. Some involvement of the extra-ocular muscles has been reported in 8 cases. The loss of vision may occur suddenly or may progress slowly over a period of several weeks. The 2 eyes may be involved simultaneously or a considerable interval of time may elapse between involvement of the first and second eyes. At times, only 1 eye is involved; but in most cases both are affected. The visual defects may vary from mistiness of vision and different patterns of field defects to complete blindness. These authors state that, although there is usually some permanent impairment of vision, some cases with even moderately severe loss for as long as 5 days may make a fairly good recovery. "The disease process must affect the arterial supply of the optic and and oculomotor nerves and the retina in varying degree, sometimes with complete thrombosis of the affected vessels and at other times cutting down the blood supply by temporary swelling of the vessel wall and later allowing eventual restoration of function, providing the degree of ischemia has not been too great." This would seem to be a logical explanation for the great variance in the subjective loss of vision and in the objective findings in the ocular fundi in the reported cases. However, in my experience, there has been little tendency to recovery of lost vision in patients with this disease. As noted by Johnson, Harley and Horton,[10] treatment with vasodilator drugs has proved to be actually harmful rather than helpful.

Cooke and his associates[3] have presented the only histopathologic studies to date of the central retinal arteries in a case of temporal arteritis. At the time of death, neither eye had light perception. The optic disks showed pallor and "primary atrophy." At necropsy, the "optic nerves showed marked softening." Sections of the central arteries of the retinas showed diffuse inflammatory changes spreading from the adventitia through the media. The lumina were obliterated by cellular fibrous tissue formed apparently during organization of a thrombus. The adventitia and media were infiltrated by lymphocytes and plasma cells and the internal elastic lamina was fragmented and calcified. A few giant cells were phagocytosing these fragments. The changes could be traced forward into the branches of the arteries.

In many of the reported cases, descriptions of the lesions in the ocular fundi have been rather vague and difficult to classify. This is not due entirely to poor or incomplete observation and recording, as a striking feature of the disease is the fact that the loss of vision in an individual case may be quite out of proportion to the visible changes in the optic disk or retina.

It is possible for sudden complete blindness to occur without visible lesions in the fundus, as in 1 eye of a patient observed by Johnson, Harley and Horton.[10] Such a case must be classified probably as a retrobulbar neuritis due to ischemia or anoxemia. On the basis of reported findings and of my observations in several unreported cases, it would seem logical to classify the lesions seen by ophthalmoscopic examination of patients with temporal arteritis into 3 groups: closures (thromboses) of the central artery of the retina or of branch arterioles, ischemic optic neuritis, and indeterminate. In the last group are included cases in which the findings are too vague to classify but are probably associated directly with the arteritis of the temporal vessels and also cases in which the changes observed in the retina may be purely incidental. Perhaps the most frequent findings is that of ischemic optic neuritis. This would seem to fit in logically with the presumed course of the disease process.

Three cases would seem to fall with fair definiteness into the group of arterial closures. In the case reported by Jennings and Comb,[9] there was complete obstruction of the central retinal artery of the left eye and "progressive obliteration of the right central retinal artery with special narrowing of its upper branches." In Case 1 of Johnson, Harley and Horton,[10] there were several ischemic areas along the superior temporal vessels of the right retina and occlusion of the upper branches of the left central artery and vein with fragmentation of the blood stream and ischemic edema at the upper pole of the optic disk. Cooke and his associates[3] state that in their Case 1 there was a "vascular block" in the left eye. The ophthalmoscopic description is rather atypical, however, since it was stated that the disk was blurred and pale, the arteries of normal size, the veins engorged and compressed, and there was a patch of exudate above the macula.

Seven cases would seem to fall into the group of ischemic optic neuritis. In Dick and Freeman's[4] Case 1, the optic disk was twice normal in size and was slightly edematous with a small hemorrhage on its surface. In Johnson, Harley and Horton's[10] Case 2, the right fundus was essentially normal. The left optic disk was mildly edematous and slightly pale, an area of whitish exudate extended out about 1 disk diameter from the temporal margin of the disk, and several small hemorrhages were present in the superior nasal retina. In their Case 3, the right optic disk was pale and slightly edematous nasally and there was an area of localized edema of the retina between the disk and the macula; the left optic disk was edematous and was rather pale in its lower temporal quadrant. In the case reported by Shannon and Solomon,[14] ophthalmoscopic examination revealed edema of the disks and retinas, threadlike arteries, engorged veins and some minute hemorrhages. Three of the cases reported by Cooke and his associates[3] seem to fall into this group. In their Case 2, in the right eye the disk was edematous and pale, the arteries were contracted, and there were in the retina 1 hemorrhage and couple of areas of edema or exudate; in the left eye, the disk was edematous and pale. In their Case 3, both optic disks were edematous and pale, the arteries were normal. In their Case 6, the right optic disk was swollen and the left normal; later both optic disks were pale and atrophic.

Nine cases must be placed in the indeterminate group; 4 of these would seem to be associated definitely with the syndrome of temporal arteries. In the case reported by Scott and Maxwell,[13] at the first observation, the optic disks were normal and the retinal vessels were essentially normal. Two months later, the right iris was atrophic and lens opacities prevented a view of the fundus; numerous small patches of exudate were present in the left peripapillary retina. No explanation was given for the loss of vision in Dick and Freeman's[4] Case 2 or in the case reported to Horton by R. A. Johnson, Cooke and his associates[3] state with refer-

ence to their Case 5 that, in the right eye, the optic disk was normal, the arteries were small but showed little sclerosis, and there were some "whitish subretinal hemorrhages" in the macular region; in the left eye, the optic disk was pale and atrophic with distinct edges, the arteries were very small and the veins large, the macular retina showed some edema.

In the 5 remaining cases in the indeterminate group, the evidence of direct connection with the temporal arteritis is not convincing. Attention has been called previously to the 2 cases of retinal phlebitis noted by Horton and Magath.[8] In Bain's[1] case, there was said to be severe photophobia and bilateral peripapillary atrophy. In Case 7 of Cooke and his associates,[3] the optic disks were normal and the retinal vessels were generally narrowed with slight arteriosclerosis. Post and Sanders[12] reported their ophthalmoscopic observations in a woman, aged 66, who had been seen 6 years before, suffering from typical arteritis of the temporal vessels, by MacDonald and Moser.[11] At this time, the ocular fundi were normal. When the patient was seen by Post and Sanders,[12] there was no visual complaint. The optic disks were normal. The arteries were markedly sclerosed and irregular in caliber. In the right retina, there were several areas of periarterial sheathing with apparent fusiform thickening of the wall through which the blood column could not be seen. Below and nasal to the macula were 3 confluent areas of silvery exudate about 1 disk diameter in extent.

The increasing number of recent case reports would suggest that the entity of temporal arteries is of more frequent occurrence than had been supposed previously. It is possible that through more general recognition of this syndrome an explanation of sudden complete or partial loss of vision in certain elderly individuals might be found in otherwise unexplained cases. Suspicion of the possible presence of arteritis of the temporal vessels should be entertained especially when the patient's history of the mode of onset of the visual loss suggests a possible closure of the central artery of the retina but the ophthalmoscopically visible lesions are atypical. When the loss of vision occurs during the phase of active involvement of the temporal arteries associated with severe headache and cordlike swelling and tenderness of the arteries and the complaint of pain or fatigue on chewing, the presence of the syndrome is rather readily recognized. At times, however, involvement of the eyes does not occur until several months after the subsidence of the acute phase of temporal artery inflammation. In such instances, a careful taking of the history is necessary to elicit the salient points which will permit of survival at the correct diagnosis.

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PHYSIOLOGY

PITUITARY-DIABETES

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Pituitary diabetes is the diabetes which persists after a short course of anterior pituitary extract. It has been divided into the temporary phase in which the hyperglycemia and glycosuria caused by the extract disappear if the extract is discontinued, and the permanent phase which continues after a more prolonged course of extract. The extracts originally used by Johns, O'Mulvenny, Potts and Laughton,[27] Houssay,[23a] and others to produce temporary diabetes and by Evans, Meyer, Simpson and Reichert,[14] and Young[50a] to make dogs permanently diabetic were crude saline extracts of the anterior lobe of beef pituitaries. Since these early studies, many investigations have added to our understanding of this form of diabetes. The recent monograph by Soskin and Levine[47] contains a valuable summary of pituitary-diabetes.

Today the 3 aspects of pituitary-diabetes which appear to deserve particular consideration are: (*A*) the identification of the hormones concerned with the metabolic activity of the pituitary; (*B*) the physiologic mechanism by which pituitary extracts, crude or refined, initiate the phenomena of diabetes; (*C*) the pathogenesis of the permanent phase of diabetes with its obvious lesions of the islands of Langerhans must be related to the preceding functional effects of the pituitary extract. The various aspects of pituitary-diabetes have been so well reviewed that it is only necessary to survey the present state of affairs.

A. Pituitary Hormones. Fraenkel-Conrat[16] recently reviewed the chemistry of the pituitary hormones, and Jensen[26] has kept the subject up-to-date. By means of the ultra-centrifuge, electrophoresis and other modern methods of protein chemistry, 5 major hormones of the anterior pituitary have been obtained in pure form. The sixth, growth hormone, has been tested in a state where only 2 proteins are present. Although a single protein with growth activity has recently been isolated by Li, Evans and Simpson,[30] its metabolic effects have not been determined. The thyrotrophic, lactogenic and 2 gonadotrophic hormones have been obtained as single proteins. In this state they manifest their respective activities with practically no overlapping of other pituitary functions. As a result, it is possible to exclude them from any major part in the diabetogenic activity of the pituitary. The exclusion of these purified hormones is in accord with Young's[50b] conclusion that the gonadotrophic and thyrotrophic substances were not diabetogenic *per se* in the dog. This means that the growth and adrenotrophic hormones are the elements largely responsible for the metabolic effects under discussion. They have been isolated so recently that extensive studies are not yet available, but Table 1 outlines the present knowledge of their action on metabolism.

The promotion of growth by growth hormone and its inhibition by adrenotrophic hormone (Table 1) have been measured by their effects on gain in weight and on epiphyseal proliferation in hypophysectomized rats,[15,38] and the authors have pointed out the antagonism between these pituitary fractions.

The lowering of the respiratory quotient by growth hormone has been reported by Greaves, Freiberg and Johns[20] and Fraenkel-Conrat.[16] No direct studies of adrenotrophic hormone on the R.Q. have been

made but Greaves *et al.* noted that a fraction potent in lowering the R.Q. had no trophic action on the adrenals. Further indirect evidence is derived from the study of adrenal cortical extract and its metabolically active steroids. Long, Katzin and Fry[32] found that the adrenal hormones depressed the R.Q. of glucose fed rats. Thorn *et al.*[49] observed this in patients with Addison's disease. Russell[42a] compared the effects of crude pituitary extract and adrenal cortical extract. She found that although both depressed the R.Q. they were quite distinct in their action on glycogen. Cortical hormone increased liver glycogen but did not directly affect muscle glycogen, whereas APE maintained muscle glycogen and did so independently of the adrenal cortex. therefore, listed as doubtful. Although both growth and adrenotrophic fractions may lower the R.Q. the evidence indicates that they do so in different ways, and this probable common effect does not prevent one from distinguishing their physiologic identity.

The ketogenic activity of crude pituitary extracts observed by Burn and Ling[7] has since then become closely associated with the growth activity. This was studied by Greaves *et al.*[20] who also noted the close correlation between the lowering of the R.Q. and ketogenic activity. The fact that the purified growth hormone, described herein, contains 2 substances[16] and the findings of Shipley,[43] and Shipley and Seymour[45] that growth and ketogenic activity are due to 2 separate principles

TABLE 1.—COMPARISON OF THE METABOLIC EFFECTS OF PURIFIED GROWTH AND ADRENOTROPHIC HORMONES

Growth	Adrenotrophic
1. Promotes growth	1. Inhibits growth
2. Lowers R.Q.	2. Effect on R.Q. doubtful
3. Ketogenic	3. Not ketogenic (inferred)
4. Nitrogen retained	4. Nitrogen unchanged or lost
5. Lowers blood amino acids	5. Amino acids unchanged
6. B.U.N. and N.P.N. unchanged	6. Increases B.U.N. and N.P.N.
7. Liver arginase unchanged	7. Increases liver arginase
8. Maintains muscle glycogen (short experiments)	8. Maintains muscle glycogen (long experiments)
9. Lowers pancreatic insulin	9. Increases pancreatic insulin
10. Slightly increases glycosuria of depan. rats	10. Markedly increases glycosuria of depan. rats

The suggestion that these substances differ in their mode of action has been supported by Bennett and Perkins,[4] and by Russell's subsequent work.[42b] Here, likewise, the glucose requirement of eviscerated hypophysectomized rats which is greater than normal was reduced by both pituitary and adrenal hormones, but the concomitant changes in glycogen led to the conclusion that the pituitary and adrenal hormones affect carbohydrate metabolism by different means.[42b] She noted that the R.Q. was depressed only in rats fed large amounts of glucose. One should add that the steroids surviving extraction or those given in pure form may not be just the same as the secretion induced by the adrenotrophic hormone. The effect of adrenotrophic hormone on the R.Q. is, of similar molecular size, should pave the way for further identification. No studies on the ketogenic action of the most purified growth preparations are at hand.

The ketogenic effect of pure adrenotrophic hormone is also unreported. The assumption that it is not ketogenic (Table 1) is based on the finding of Shipley and Fry,[44] that adrenal cortical compounds do not stimulate ketosis.

The well-known ability of pituitary extract to promote nitrogen retention has been identified particularly with the growth hormone by Marx, Magy, Simpson and Evans.[37] They did not use purified adrenotrophic hormone but adrenal cortical extract had no such effect. In other investigations, summarized by Long, Katzin and Fry,[32] nitrogen excretion was

increased by the adrenal cortex and the contrast expressed in Table 1 seems to be a logical assumption. The different effects of these pituitary principles on nitrogen metabolism receives further support from the study of their action on the nitrogenous elements of the blood. Fraenkel-Conrat, Fraenkel-Conrat and Evans[17] reported the changes listed in Items 5 and 6 of Table 1, using the purified pituitary fractions. In addition, Fraenkel-Conrat, Simpson and Evans[19] distinguished these principles by their action on liver arginase.

The relationship of the pure principles to muscle glycogen has been measured by Herring and Evans.[22] Although both maintain muscle glycogen, they do so under different conditions. These results supplement the studies of Russell[42a,b] already cited and all investigations are in agreement with the concept that the growth and adrenotrophic effects influence carbohydrate metabolism by different mechanisms.

The effect of pituitary hormones on the insulin content of the pancreas has been measured by Fraenkel-Conrat, Herring, Simpson and Evans.[18a,b] This effect may well be secondary to the alteration of the metabolic constituents because it is comparable to the changes induced by diet.[3]

Finally, the influence of growth hormone on the glycosuria of partially depancreatized rats has been recorded by Marx, Anderson, Fong and Evans.[36] They found that there was a definitely increased glycosuria in 5 of 13 rats tested, the other animals having only slight changes. Compared to this slight response the powerful glycosuric action of adrenal cortical compounds[32] and the glycosuric action of crude adrenotrophic preparations provides, in the Reviewer's opinion, a contrast between these hormones.

Summarizing this section, the tentative conclusion is that when purified hormones are used there is a good physiologic distinction between the 2 fractions which are responsible for most, if not all, of the diabetogenic activity of the whole gland. The extent to which 1 of these principles is diabetogenic cannot be determined until larger quantities of the pure hormones are available for use in dogs. The absence of hyperglycemia from the activities listed in Table 1 is noteworthy. This action of pure growth hormone is not recorded, but Long, Katzin and Fry[32] reported an increase in blood sugar from 114 to 154 mg. per 100 cc. when rats fed glucose were treated with cortical extract. Still more striking was Ingle's[25a] demonstration that 11 dehydro - 17 - hydroxycorticosterone caused severe hyperglycemia and glycosuria in normal, fed rats. More recently Ingle, Winter, Li and Evans[25b] have produced glycosuria in normal rats by pure adrenotrophic hormone. At present one can only admit that all diabetogenic activity has not yet been identified with a single hormone, although the means of doing so appear to be at hand.

There is no quantitative assay for diabetogenic activity and possible differences in the methods used must be kept in mind. Thus, a rat deprived of 95% of its pancreas may be so sensitive that an increase in glycosuria may result from small doses of growth or adrenotrophic hormones, and from what has been said these results might be due to either underutilization or overproduction of carbohydrate. Such sensitive animals are suitable preparations with which to eliminate the clearly inactive fractions. On the other hand, a much more potent hormone is needed to produce diabetes in the normal dog and there is no evidence that any pure hormone will do this. In addition, the numerous functions which may share in the diabetogenic effect demand multiple assay methods. In Table 1, Items 2, 3 and 10 are diabetogenic properties of the growth hormone and most of the effects of adrenotrophic principle are appropriate to a diabetogenic hormone. Therefore, several of the present methods of assay will be required to elucidate the rôle of the pituitary principles concerned with metabolism.

B. Diabetogenic Mechanisms. Pending further investigation of purified ex-

tracts it is well to reëxamine the action of crude extracts in order to appraise the physiologic mechanism by which the anterior pituitary is diabetogenic. For the purpose of discussion, it will be assumed that the hormones act for the welfare of the peripheral tissues, and hence the action of the anterior pituitary will be reviewed with respect to its action on muscle. The control of muscle glycogen will be the focal point to which the other effects of the gland will be referred. The influence of the hormones on blood sugar, liver glycogen, and ketogenesis will be briefly related to the changes occurring in the muscles.

when the various studies[24,31,42a,b,50b] are reviewed it seems clear that the anterior pituitary maintains glycogen or permits its increase. This does not imply that it promotes glycogen deposition by the same mechanism or to the same degree as insulin. Both insulin and pituitary extract can increase the glycogen level but this increase is always accomplished in the presence of both hormones. In such a system, either insulin or the anterior pituitary may be in excess, but the hormone which is relatively inactive still exerts some influence. This is no new observation. For example, Soskin[46] notes that insulin is "but one factor in a bal-

TABLE 2.—SOME EFFECTS OF INSULIN AND ANTERIOR PITUITARY EXTRACT (APE) ON MUSCLE GLYCOGEN

			Other effects				Schematic effect	
Procedure	Hormones present	Muscle glycogen	Liver glycogen	Blood sugar	Protein to COH	Keto-genesis	Glucose to cell	Glucose from cell
Glucose to normal	Insulin APE	+	+	+	−	−	+	−
Insulin to normal	Insulin APE	+	−	−	−	−	++	−
APE to normal	Insulin APE	+	+	+	+	+	−	−−
Pancreatectomy	APE	−	−−	++	++	++	−−	−
Hypophysectomy	Insulin	−	−	−	−−	−−	+	++
Houssay animal	"None"	−	−	+	−−	−−	−−	++

+ = increased; − = decreased.

Table 2 shows the effects on muscle glycogen of the administration and of the removal of insulin and the anterior pituitary. The response to glucose needs no comment and the action of insulin on muscle glycogen is well known. The effect of anterior pituitary extract on muscle glycogen has been studied by Houssay, Biasotti and Dambrosi,[24] Young,[50b] and Long.[31] The data of Long on the increased muscle glycogen in mice when glucose and pituitary extract were administered have been compared with the increment found by Bridge[6] when glucose and insulin were given. It is true that in some experiments the administration of pituitary extract has been associated with a fall in muscle glycogen. However, anced endocrine system," and Young[50c] cites the complex action of growth hormone as an "example of the duality of many biologic systems which may be constituted from two or more balanced reactions." The antagonism between the anterior pituitary and insulin has often been outlined and is in part shown in Table 2 in the column listing "other effects." However, the fact that the opposing hormones act together to produce a common effect on the muscle has received less attention than the contrast between the hormones. One may postulate that the primary purpose of insulin and the anterior pituitary is to maintain the "nutrition" of the muscles (tissues) at all times. The action of insulin seems to

dominate when the animal is fed; the action of the anterior pituitary is essential during fasting.

The last 3 lines of Table 2 show that muscle glycogen is lowered when one or both hormones are removed. After hypophysectomy muscle glycogen falls rapidly during fasting although the fed hypophysectomized animal maintains its muscle glycogen fairly well.[23b,24] The fall in muscle glycogen in depancreatized animals is slight,[10,33a] as is the case in the more variable glycogen levels of the Houssay animal.

The list of other effects (Table 2) could be amplified but the brief data given show the variety of adjustments in the metabolism of protein, fat and carbohydrate which crude pituitary extract may make, largely due to the intervention of the liver. The + and − signs indicate tendencies to increase or decrease which may be more or less apparent according to the experimental conditions. Glycogen levels depend on the availability of glucose as well as on the action of the hormones. The ingestion of glucose, the mobilization of glucose from endogenous sources and, in the absence of insulin, the high level of blood sugar are recognized factors in the formation of muscle glycogen which in the table has been related only to hormones. In this connection the low glycogen levels of the phlorhizinized animal[39] in which both hormones are present, and the lower glycogen in fasted hypophysectomized animals than in Houssay animals with hyperglycemia, are examples of the rôle played by the blood sugar level in the maintenance of muscle glycogen.

The last column of Table 2 attempts a schematic explanation of the changes in muscle glycogen under the various conditions. In the normal animal, an increased muscle glycogen is a common effect of insulin and of anterior pituitary extract. At the same time, as indicated under other effects, blood sugar, liver glycogen, gluconeogenesis and ketogenesis are influenced in an opposite manner by these hormones. Under these circumstances the Reviewer assumed, quite arbitrarily, that the hormones might act on or in the muscle cells. In this tissue, glycogen storage would logically result from a positive or favorable balance between the supply and utilization of glucose within the cell. This concept permits an explanation of the effects of insulin and the anterior pituitary on muscle glycogen under the conditions outlined. One may cite the overworked analogy of the reservoir and suppose that insulin opens the inlet and that pituitary extract acts to close the outflow. (In such a scheme the liver would be an emergency reservoir just up stream.) The possible combinations maintaining a high level in the "muscle reservoir" have been expressed by arrows and + or − signs. Plus signs indicate an acceleration, minus signs a retardation of the reactions indicated by the headings. It is necessary to recall the danger of such simple analogies and to remember that the transfer of glucose to and from the cell may result from the passage of glucose from the capillaries, or through the cell wall, or its utilization in any of the numerous chemical reactions concerned with the storage or oxidation of glucose in the cell. Finally, the primary assumption that these hormones act on the tissues will be questioned by those who regard changes in glycogen as automatic responses to the availability of glucose which is provided and regulated by the liver *via* the blood. The fact that insulin and pituitary extract appear to act quite differently on the phenomena mediated by the liver suggests that their action on the muscles is peripheral. This does not deny the importance of a supply of glucose in these reactions as has been mentioned.

The preliminary report of Price, Cori and Colowick[40] on the action of anterior pituitary extract and insulin *in vitro* forecasts a new understanding of this whole subject. They studied the reaction: glucose + adenosine triphosphate → glucose-6-phosphate + adenosine diphosphate

which is catalyzed by hexokinase. They found that this reaction is inhibited by an anterior pituitary extract containing growth and ketogenic activity.[20] Insulin releases hexokinase from the inhibition of the anterior pituitary although insulin by itself has not been observed to alter hexokinase activity. Because the formation of glucose-6-phosphate is a reaction common to the oxidation of glucose and to the formation of glycogen these results support the physiologic estimation that pituitary extract inhibits glucose oxidation. The location of the pituitary extract *versus* insulin antagonism at a single chemical reaction would seem to nullify the "explanation" in the last column of Table 2. Nevertheless, the common action of insulin and the anterior pituitary on glycogen levels in the intact animal, indicated in Table 2, appear to require for their explanation more than is now known of the hexokinase reaction. In like manner the insulin sensitivity of the hypophysectomized animal is not accounted for by an action of insulin exerted only in the presence of the anterior pituitary. It is too early to evaluate fully the significance of these important observations.

Summarizing this section, emphasis has been placed upon the effect of the anterior pituitary in a hormonal system rather than its isolated action. The possible influence of hormones on the tissues, rather than their action on supply depots and the liver, has been assumed. Although this outline requires correction and enlargement it has been offered as an introduction to the conditions under which pituitary extract produces diabetes.

It is now agreed that the anterior pituitary is a factor in maintaining the organism during fasting. For this purpose it achieves a fairly definite quality and quantity of the metabolic mixture. The administration of pituitary extract to fasted animals adds but little to the effect of starvation and does not produce diabetes.[23a] However, it accelerates the adjustment to fasting so that the impaired sugar tolerance which results in 2 to 3 weeks of fasting is accomplished in 2 to 3 days by pituitary extract, and it prevents the usual readjustment made by the fasting animal when carbohydrate is fed. Dohan and Lukens (unpublished) have given pituitary extract to fasting animals which were partially depancreatized and in only 1 or 2 with very small pancreatic remnants was a slight glycosuria produced. In the fed animal, either intact dog or partially depancreatized cat, these extracts readily produced diabetes. Under these circumstances it seems that large doses of pituitary extract may fix or set the metabolism in the pattern needed for fasting. When such a low carbohydrate régime is made "rigid" by extract, most of the carbohydrate food ingested is in excess of the amount which can be utilized, so that hyperglycemia and glycosuria result. In the early stage of extract treatment and lacking knowledge of the animals' endogenous metabolism, the factor of overproduction seems to be attributable to food intake rather than to the quantity mobilized by the pituitary from body depots.

Dohan, Fish and Lukens[11] have observed the nitrogen retention of crude pituitary extracts prior to the development of severe glycosuria. From these experiments, the data in Table 3 are presented to show that nitrogen retention occurs in the first 3 days of pituitary treatment when hyperglycemia develops, but before the islands are visibly injured. This action of diabetogenic extracts on nitrogen metabolism has also been shown in rats by Cuthbertson, Webster and Young.[9] According to Lee and Schaffer,[29] and Lee[28] the gain in weight during APE is due to nitrogen and water retention and the body fat remains constant or diminishes somewhat. Although protein food is part of the diabetogenic régime, the retention of nitrogen suggests that overproduction of glucose from protein is not unrestricted and probably is not the sole factor accounting for glycosuria. The prevention of hyperglycemia by an isocaloric high fat diet suggests that fat is not a source

of glucose under these conditions. It is, therefore, the reviewer's opinion that pituitary extract (growth, ketogenic or both) suppresses carbohydrate oxidation and that this action is one essential of its diabetogenic effect. The other essential is food from which glucose is produced at a rate for which the adrenotrophic hormone is apparently necessary.

At present, the studies with pure pituitary hormones and those with crude extracts are in fair agreement. They all indicate that the diabetes produced by crude anterior pituitary extracts is the result of an inhibition of the utilization of carbohydrate and of its simultaneous mobilization from food plus endogenous sources. The conclusion that 2 principles —the growth and adrenotrophic hormones—are necessary to diabetogenic action remains valid until more knowledge of the pure hormones is available.

persisted from 4 to 6 weeks in the dog, the island lesions which began as hydropic degeneration progressed to atrophy. On the other hand, partial pancreatectomy unaccompanied by diabetes was not harmful to the islands. The relationship between pancreatic deficiency and diabetes, and the association of diabetes and injury of the islands of Langerhans was recognized before the discovery of insulin.

In like manner, diabetes has been produced by anterior pituitary extract only when sustained hyperglycemia has been effected. The following observations illustrate this point. Some species, such as the mouse, rat and guinea pig, are quite insensitive and do not respond to pituitary extract with hyperglycemia and glycosuria. These animals do not develop lesions of the islands. Moreover, in those species which are susceptible there are individual animals which become refrac-

TABLE 3.—CHANGES IN WEIGHT AND NITROGEN EXCRETION AT ONSET OF PITUITARY-DIABETES

	Control—3 day periods			APE—First 3 days		
				Average urine		
Dog No.	Initial weight (kg.)	Gain or loss in weight (kg.)	Average urine N (gm./day)	Nitrogen (gm./day)	Glucose (gm./day)	Gain in weight (kg.)
P–16	8.35	+0.15	15.67	11.53	2.7	0.40
P–19	5.85	−0.20	14.90	9.80	3.7	0.35
P–22	8.80	−0.10	13.30	9.40	18.8	0.25
P–14	10.45	+0.80	23.60	18.10	1.1	0.85
P–13	16.80	−1.10	6.63	7.60	0	−0.46

The diets varied but for each animal the diet was the same in the control and extract periods. Dog P 13 was fasted for both periods.

This leads to the next question: What is the mechanism by which the lesions of the islands and the permanent phase of diabetes is produced?

C. THE PATHOGENESIS OF THE PERMANENT PHASE OF PITUITARY-DIABETES. Long before the era of pituitary-diabetes it had been ascertained that partial pancreatectomy, if sufficiently extensive, was accompanied by diabetes. Begun by Minkowski and culminating in the work of F. M. Allen, these investigations showed that partial pancreatectomy did not cause lesions of the islands of Langerhans except when hyperglycemia had been present. If this type of diabetes tory to pituitary extract. After the hyperglycemia caused at first by the extract has subsided, these refractory animals show no island lesions even though the administration of extract is continued. A third point, already mentioned is that pituitary extract is ineffective in fasted, fat-fed, or insulin-treated animals.[21] In all of these conditions which prevent the development of hyperglycemia there is no damage to the pancreatic islands.

The prevention of diabetes in the presence of an effective diabetogenic procedure deserves further comment. Allen[1a] showed that "partial pancreatectomy to the extent sufficient for severe diabetes is

followed by no hydropic degeneration when the diabetes is thoroughly controlled by diet." Bell, Best and Haist[2] have shown that the content of insulin per gram remained normal in pancreatic remnants when diabetes was absent but that it was reduced in similar remnants of diabetic animals. Lukens and Dohan[34] have confirmed the prevention of pituitary-diabetes[21] by the use of isocaloric combinations of meat and fat in partially depancreatized cats. This is recalled not only in connection with the rôle of hyperglycemia in pathogenesis, but to emphasize the need for protein or carbohydrate food in conjunction with pituitary extract, if hyperglycemia is to be maintained. Finally, the occurrence of island damage by pituitary extract has been avoided by the too small to maintain normal metabolism. Their only animal with atrophy of the islands failed to improve under insulin treatment.

In dogs the characteristic lesion of the islands of Langerhans in the permanent phase of pituitary-diabetes is atrophy.[11,41] This is so because it takes 4 to 6 weeks of injection to establish permanent diabetes and, as Allen[10] showed, the early hydropic degeneration is followed by atrophy within this period. Lukens and Dohan[34] used partially depancreatized cats without spontaneous diabetes in studying pituitary-diabetes. As in the dog, permanent diabetes could be produced by 4 to 6 weeks of extract treatment. Unlike the dog, hydropic degeneration of the beta cells lasted for the

TABLE 4.—PITUITARY-DIABETES IN THE CAT
Treatment Begun Within 3 Months

		Effects of therapy				
		During treatment		After treatment		
No. expts.	Therapeutic measure	Blood sugar	Glucose retained	Island structure	Island function	Glucose retained
2	200 gm. beef reduced to 100 gm. daily	Becomes normal	Decreased	Largely restored	Recovered	Increased
9	Insulin		Increased		Recovered	
1	Adrenalectomy		Increased		Improved?	
8	Phlorhizin		Unchanged		Recovered	

simultaneous administration of phlorhizin.[35] Phlorhizin given at the same time as pituitary extract abolished the hyperglycemia, although glycosuria and ketonuria persisted. Biopsies showed normal islands when the blood sugar was normal and marked hydropic degeneration after the period of hyperglycemia.

Having mentioned the factors involved in the production and prevention of experimental injury to the islands the parallel studies on the recovery of island damage may be reviewed. Soon after the discovery of insulin, Copp and Barclay,[8] and Bowie[5] observed morphologic recovery of hydropic islands in partially depancreatized dogs treated with insulin. In spite of the restoration of the islands, recovery of the animals was impossible because the pancreatic remnants had been originally first 3 months of glycosuria. After 3 months of diabetes, atrophy of the islands ensued as in the dog. Thus, the cat provided a preparation having a stable diabetes with a reversible type of lesion for 1 to 2 months after the cessation of pituitary extract. Within the first 3 months of the disease cats with pituitary-diabetes have been treated, and the islands restored, by the 4 procedures which Table 4 outlines. The anatomic repair of the islands and the functional recovery of the animals have been reported[33b,34,35] and discussed. In addition, the whole subject of the pathology of experimental diabetes has been fully reviewed by Duff.[13] The anatomic recovery following dietary and insulin treatment is quite in line with the earlier observations in partial pancreatectomy. However, as our animals

were not diabetic prior to pituitary treatment the restoration of their originally adequate insular tissue was accompanied by physiologic recovery from the diabetes. Recovery after insulin therapy was achieved in animals with severe diabetes in which 90% of the available glucose of the diet was excreted in the urine. Recovery by means of reduction in diet was only possible when the diabetes was mild and could be controlled by diet. Phlorhizin was effective in severe diabetes and deserves some comment.

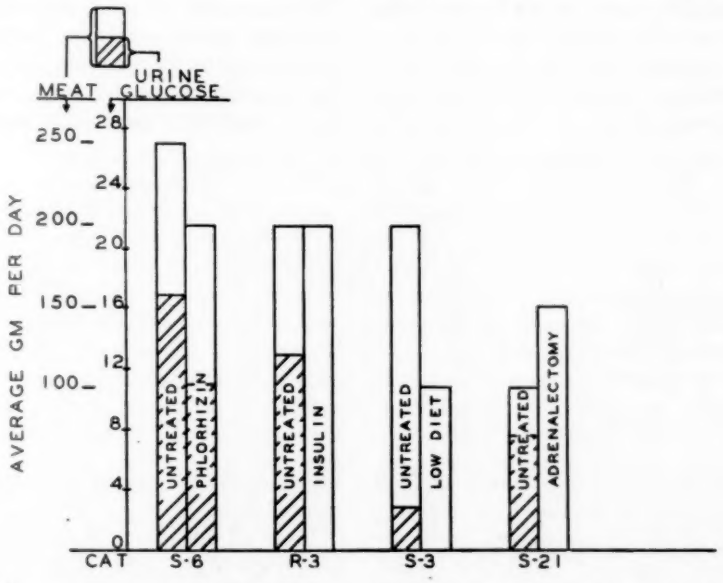

FIG. 1.—Average meat ingested and glucose excreted in 4 types of treatment of early pituitary-diabetes. The daily averages were calculated from 6 day periods before treatment and after treatment had been established. The scales have been so arranged that grams of urinary glucose also represent the available glucose of the diet (D:N = 3.65). Thus, the height of the columns, above the hatched area indicating urinary glucose, shows the "glucose retained." The subsequent recovery of the islands has been described (text).

Allen[15] and others have demonstrated the ability of phlorhizin to lower the blood sugar level in diabetes. A comparison of the actions of phlorhizin and insulin has been made[35] which may be summarized as follows. Each drug lowers the diabetic blood sugar and each one brings about recovery of the islands when the lesions are reversible. Except for these 2 similarities the comparison reveals only contrasts between insulin and phlorhizin. Phlorhizin impairs the reabsorption of glucose by the kidney; insulin has no such action. Phlorhizin increases protein catabolism as measured by nitrogen excretion; it increases ketogenesis and decreases the amount of food assimilated, at least by mildly diabetic animals. In diabetes, insulin has the opposite effect on these processes. The less certain results of phlorhizin in decreasing carbohydrate oxidation and lowering glycogen stores are probably secondary to the loss of glucose. They differ from the conspicuous and probably primary action of insulin on the oxidation and storage of glucose. Admitting the controversial elements which pervade such a summary, the contrast between phlorhizin and insulin, except for their effect on the diabetic blood sugar levels adds further evidence that the normal level of blood glucose is responsible for the recovery of the islands of Langerhans.

Returning to another aspect of the 4

therapeutic measures presented, Figure 1 shows examples of the effect of each procedure on the amount of food assimilated. The scales for the amount of meat eaten and for urinary glucose have been so arranged that grams of glucose correspond to the available glucose of the meat diet when a D:N ratio of 3.65 is used. The total height of the column, therefore, gives the conventional available glucose and the open portion the glucose retained. The writer shares the opinion that the D:N ratio is a constant arbitrarily selected from variable observations.[46] For this reason it may be well to make the comparison between food intake and urine glucose without making assumptions about the interconversions of the foodstuffs.

Table 5.—Comparison of First and Second Induction of Diabetes

Cat No.	Period of pituitary treatment: Diabetes I (days)	Period of pituitary treatment: Diabetes II (days)	Dosage of anterior pituitary glands: Diabetes I (gm.)	Dosage of anterior pituitary glands: Diabetes II (gm.)
R–10	25	12	60	20
R–12	12	6	33	15
R–21	13	13	36	18
T–7	24	30	42	33
T–8	22	10	50	10
Average	19	14	44	19

The point is that the amount of food assimilated may be increased (R–3, S–21), decreased (S–3) or unchanged (S–6) by the various types of treatment which ultimately lead to the restoration of the islands of Langerhans. It is necessary to emphasize the fact that the responses *during treatment*, shown in Figure 1 were followed by the full utilization of the usual diet without diabetes after treatment was stopped, as has been shown in the original reports.[34,35]

The criteria of recovery in these experiments have been freedom from glycosuria, a normal level of blood sugar and the ability to gain weight on the diet taken prior to treatment. In addition, a few animals have had sugar tolerance tests (1.8 gm. glucose per kg. orally). Of 7 cats tested after recovery, 5 had normal and 2 had mildly diabetic curves. These tests are somewhat variable in cats but they correspond approximately to the results obtained in remissions of diabetes in man.[33a] The conclusion seems to be that recovery is somewhat limited, and this is confirmed by another type of experiment. In 5 recovered animals, diabetes was induced a second time by pituitary extract. Table 5 shows that much less pituitary extract was needed to make these cats diabetic again. It is unlikely that the loss of pancreatic tissue from biopsy or scarring was enough to account for this. The suggestion is made that little more than minimal essential function is regained under treatment so that these islands readily succumb to another stress. These results remind one of the frequency with which infection causes exacerbations of clinical diabetes.

From all this it is clear that both partial pancreatectomy and anterior pituitary extract have caused lesions of the pancreatic islands only in the presence of hyperglycemia. Both procedures lead to hydropic degeneration of the beta cells after 1 to 2 weeks of hyperglycemia. If treated early, the islands of Langerhans are restored by 4 measures (low diet, insulin, adrenalectomy and phlorhizin), which lower the blood sugar to normal levels. The contrast between phlorhizin and insulin, except for their effect on the blood sugar, has provided further support for the hypothesis that hyperglycemia plays a part in the pathogenesis of diabetes. The preliminary nature of this hypothesis is apparent when one surveys a few of the facts which do not seem to

conform to it. (*a*) Hydropic degeneration does not occur in the diabetic rat and more species should be studied before we finally interpret the results which appear so clear in the cat and dog. (*b*) Mild diabetes may persist for long periods in animals and man without rapid progression in the severity of the disease. This stability of mild diabetes is in contrast to the severe glycosuria with a similar hyperglycemia produced by pituitary treatment. (However, in mild diabetes the early island lesions progress to irreversibility as was noted.) (*c*) Two case reports, 1 by Sprague, Priestley and Dockerty,[48] and 1 by Duncan, Semans and Howard,[12] compel a cautious attitude toward the rôle of hyperglycemia. In both cases diabetes mellitus of 3 years duration was followed by complete recovery after the removal of adrenal tumors. By comparison with the animal experiments, these patients ought to have had irreversible damage of the islands.

After weighing the pros and cons the bulk of the evidence seems to favor the hypothesis that the level of blood glucose is a factor of importance in the pathogenesis of diabetes. It may well be that hyperglycemia has to be associated with some other factor to produce island damage. If this be so, present knowledge suggests that hyperglycemia may help us to trace the unknown elements in pathogenesis much as the chemist uses a labelled atom to trace substances in the body.

Summary. According to studies done so far, no single purified pituitary hormone contains all the diabetogenic properties. Growth hormone is ketogenic and by the present limited criteria inhibits the utilization of carbohydrate. The adrenotrophic hormone mobilizes protein and carbohydrate and so is necessary for the phenomena of overproduction observed in diabetes. On theoretical grounds, maximum diabetogenic activity would result from a combination of these hormones, and this seems to be the case when crude extracts are employed. The mechanism of action of crude pituitary extracts is still poorly understood. However, this action includes evidence of the suppression of carbohydrate utilization and of overproduction under certain conditions. The effects of crude extract on glycogen storage, protein metabolism and fat metabolism compose a picture not incompatible with the gross observation that protein or carbohydrate food is necessary for the greatest diabetogenic effect. The suggestion has been made that the degree of glucose production and utilization needed by the fasting animal is so established or "fixed" by pituitary extract that carbohydrate or protein food cannot be metabolized in the normal manner and hence leads to hyperglycemia.

If this hyperglycemia is sustained long enough, at least in the dog and cat, the islands of Langerhans undergo a sequence of structural changes resulting in irreversible damage and permanent diabetes.

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SURGERY

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ORGANIZING HEMOTHORAX—A CLINICAL ENTITY

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1. Introduction. The significance of organizing hemothorax has only recently been recognized and the methods of diagnosis and treatment are largely developments of the recently terminated World War II. That the condition not infrequently existed unrecognized prior to this time can scarcely be doubted. Personal communications from both military and civilian pathologists and others who have had occasion to deal with traumatic disabilities of the chest confirm the fact that the condition was not uncommon, but its nature and clinical significance was entirely missed or imperfectly understood. Seen at operation or autopsy in the years prior to its elucidation, organizing hemothorax was usually misdiagnosed as organizing empyema or simple pleural thickening and so treated and summarily dismissed. The reason for this oversight lies in part with the frequently repeated statement in surgical texts that blood in the pleural cavity clots but rarely, and therefore the basic factor in the development of organizing hemothorax, that is, the presence of clot in the pleural cavity as the underlying pathologic cause of the condition received little attention. With the high incidence of penetrating wounds of the thorax in World War II, however, together with the material advances in the technique concerned with thoracic surgery, the existence of the condition as a more or less definite clinical entity was established and methods for its diagnosis and treatment progressively developed. Much concerning this disability remains to be clarified, but, as previously stated, the basic principles in its recognition and therapy have already been more or less established. To be convinced of the relative newness of the subject, it is only necessary to search the surgical literature for data concerning it. Such material is extremely scanty and that now available is largely derived from personal communications. The literature concerned with the condition will doubtless continue to grow from both civilian and military sources because of its importance in the special field of thoracic surgery.

There can be little doubt that the condition of organizing hemothorax is of considerable import clinically. Its recognition and successful treatment in World War II has resulted in the restoration to full active military or civilian life hundreds of individuals who would otherwise have remained permanently incapacitated, or at best in a state of restricted activity. To what extent this lesion contributed to the number of those with permanent pulmonary disability during and after World War I is a matter of conjecture, since statistics dealing with the same are not available, but the probability is that it was not a negligible factor.

2. Definition. Organizing hemothorax may be defined as a condition usually produced by trauma to the thoracic cavity or its contents in which there exists within the pleural space a clot formed by the resultant hemorrhage which subsequently

undergoes progressive organization by the ingrowth of fibrous connective tissue from the adjacent pleural structures.

3. Incidence. Organizing hemothorax can occur at any age, but since it is practically always produced by trauma to the thorax or its contents, its incidence is far greater in the age groups at which trauma is the most common or during the period of greatest physical activity. It is therefore most common between the ages of 20 and 40. In civilian as well as military life its incidence is far greater in males than in females, and it is far more prone to occur in individuals in the lower strata of society, since strife leading to violence is far more frequent in lower social scales.

The highest recorded number of cases occurred in the period 1943–45 during the interval of active warfare in the European Theater, particularly during the Italian campaign. That period corresponded to the time during which the heaviest fighting occurred and in which the greatest number of casualties were sustained. This was to be expected as the cause of organizing hemothorax is, as previously pointed out, traumatic in practically all instances. Complete statistics concerning the specific wounds of this warfare are not as yet available, but a reasonable estimate of the incidence of cases of penetrating wounds involving the chest which survived to receive definite treatment compared to the total of all treated wounds is in the region of 1 to 2%. Of these, practically all showed a greater or less degree of hemothorax; and of the hemothorax cases between 25 and 40% showed the criteria for establishing a diagnosis of organizing hemothorax. This is in marked contrast to the incidence of the lesion following hemothorax incurred by thoracic injuries in civilian life. The incidence of hemothorax following trauma in civilians is far less. Unfortunately such figures have not as yet been accurately formulated from existing data; but it is the opinion of the author as well as others who have frequently to deal with thoracic trauma that, in civilian practice, organizing hemothorax occurs in considerably less than 3% of hemothorax cases. The reason for this, as will be noted later, lies in the difference in the ordinary types of wounds encountered in military and civilian activity.

4. Pathogenesis and Pathologic Physiology. It has just been stated that organizing hemothorax is practically always secondary to some well-recognized form of chest trauma which produces hemorrhage into the pleural cavity. Usually the trauma is severe, such as penetrating wounds made by sharp instruments or missiles, and crushing injuries to the chest wall. In practically all instances there is damage of some extent to the contained lung. The essential feature, however produced, is an intrapleural hemorrhage arising either from the vessels of the thoracic wall mediastinum, or from the traumatized pulmonary parenchyma. The extravasated blood in the pleural cavity gravitates to the most dependent portion of the pleura and subsequently undergoes partial or complete clotting forming a more or less solid jelly-like mass which compresses the adjacent lung tissues against the chest wall or mediastinum. Any degree of compression of the lung may occur. The entire lung may be involved and thus rendered atelectatic and airless, but more usually the process is confined to one lobe, in most instances the lower. The clot thus formed thereafter undergoes a process of invasion by connective tissue fibers from the surrounding pleural structures and ultimately forms a completely organized clot which effectively and permanently impairs activity in the lung which it overlies. The end-result, so far as the pulmonary function is concerned, is a marked reduction in the amount of functioning lung tissue. The vital capacity is reduced in direct proportion to the extent of pulmonary tissue involved which is in turn determined by the extent of the clot. Since the lung in the affected area cannot aërate, the pulmonary reserve, as indicated by the vital capacity, is diminished. The diminution in the pulmonary reserve

may not be immediately apparent under ordinary circumstances, that is, under the normal circumstances of a sedentary existence; but it is noticeable when an increased physiologic demand is put upon the respiration, as in unaccustomed physical exertion. The deficiency becomes noticeable by a more rapid onset of the symptoms associated with anoxia, namely dyspnea, fatigue, and a slower rate of respiratory recovery.

thorax, then, is an intrapleural, extrapulmonary hemorrhagic clot of variable dimensions, which as time progresses becomes organized by the ingrowth of fibrous connective tissue from the adjacent pleural structures (Figs. 1 and 2). The sequence of events is first the extravasation of blood usually in the form of a single massive hemorrhage into the pleural cavity. Sometime thereafter the fluid blood becomes clotted and the process of

FIG. 1.—Case G. A. Photomicrograph (40 ×) showing section of organizing clot removed at operation. Note mass of clot already organized by dense fibrous tissue (right).

It will be seen that whatever anoxia is produced by the lesion is primarily of the anoxic type, and is responsible for the presence of the associated signs and symptoms. Obviously, the severity of the symptoms will be in proportion to the degree of anoxia produced by pulmonary compression from the clot. As in other disabilities affecting all or a portion of the lung, the remaining functional lung tissue compensates to a certain extent for the loss of the affected part. But this compensation can at best be only partial, so that the physiologic limit of the respiratory reserve (measured by the vital capacity) is lowered considerably and permanently, and remains so unless the condition is corrected.

The essential lesion of organizing hemo-

connective tissue ingrowth begins. This ingrowth is not uniform throughout the clot, proceeding faster in some areas than in others, so that unless the clot is very small, multiple loculi may be formed containing shreds and strands of fibrin and laked blood. Ultimately it is probable that the entire mass becomes fibrosed and undergoes contracture.

The microscopic picture of the essential lesion depends upon the age of the condition. Initially the process shows only the clot with a network of fibrin strands in which the cellular elements are embedded lying between the pleural layers of mesothelium. Later the typical process of inflammation is present with the appearance of polymorphonuclear cells, microphages, lymphocytes, phagocytic dissolu-

tion of the fibrin, and laking of the red blood cells. Still later, as organization progresses, there is proliferation of the fibrous connective tissue cells into the clot mass together with ramification of the endothelium of the blood-vessels. Ultimately the connective tissue cells begin to show contracture of their processes and the greater part of the mass assumes the appearance of solidly organized connective tissue. If, as very frequently happens, the clot contains infectious material (bacteria or foreign bodies), the inflammatory reaction is greatly accentuated and the inflammatory products

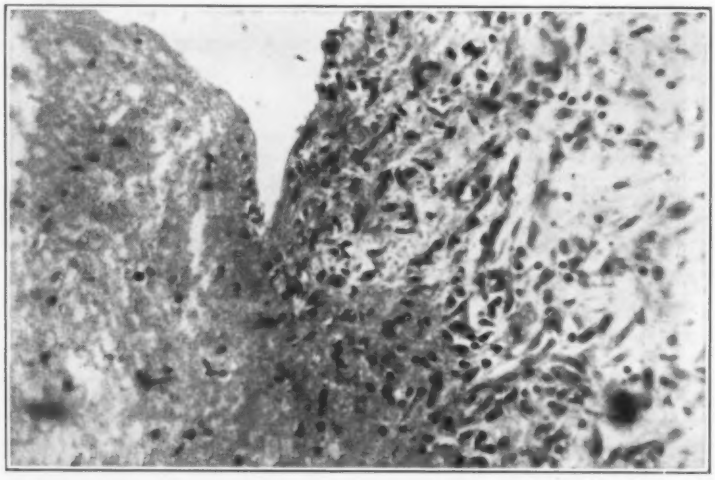

FIG. 2.—Case G. A. Photomicrograph (97 ×) showing section of organizing clot removed at operation. Note ingrowth of fibrin (left) by connective cells (right).

greatly increased. It is probable that the process of organization of the clot is accelerated by the presence of inflammatory agents and the amount of associated fibrosis is augmented. The amount of inflammatory process present will depend largely upon the character and number of bacterial agents producing it.

Practically all penetrating wounds of the pleura are contaminated since the agents producing them are rarely sterile or even surgically clean. However, infection resulting from such contamination occurs in considerably less than half the cases. The bacterial agents producing intrapleural infections in cases of organizing hemothorax vary somewhat with the conditions under which the wound is inflicted, but in the main are fairly constant. *S. aureus* is the most frequently recovered, and next in order B. hemolytic streptococcus and colon bacillus. Mixed infections are relatively frequent, depending, as stated previously, upon the conditions of infliction and the type of the wound.

Precisely what factors determine whether or not slot formation will occur in blood extravasated into the pleural cavity are not known. It is probable that the process is determined to a large extent by the presence of contaminating agents and the character of the wound. There is no doubt that intrapleural clotting occurs with far greater frequency in instances of thoracic trauma encountered incident to warfare than in those observed in civilian life. In the former, the wounds are considerably more extensive, involving as a rule larger areas of lung and chest wall, and gross contamination with pathogenic organisms and the presence of foreign bodies are much more frequent. Intrathoracic wounds in civilian life are usually inflicted with relatively cleaner agents of penetration which, as a rule, involve

only small areas of pleura and pulmonary parenchyma. It is probable that extensive injuries of the lung parenchyma increase the probability of intrapleural clotting by the liberation of thromboplastic substance which the lung is known to contain in considerable quantity. The rôle of contaminating agents in the clotting process has been subject to much speculation, and in general the consensus of qualified opinion is that certain bacterial contaminants predispose to premature clotting. Frequently mentioned in this connection is the most common contaminant isolated from cultures of intrapleural clot, and (2) localizing, resulting from compression of the affected lung.

The constitutional manifestations of organizing hemothorax are indistinguishable from those incident to pleural reaction from liquid blood or infection in the pleura and are in general those incident to any inflammatory reaction. There is usually a moderate elevation of the temperature (99° to 101° F.) with a proportional concomitant rise in the pulse rate and respiration. Leukocytosis of some degree is present and there is more often than not an associated anemia of the hypochromic microcytic variety. These

A. *B.* *C.*

FIG. 3.—Case G. A. *A*, Roentgen ray showing postero-anterior view of chest on October 22, 8 days after injury. Aspiration at this time showed blood contained in pleura to be unclotted. *B*, Roentgen ray showing postero-anterior view of chest on November 8, 23 days after injury. Repeated aspiration showed no recovery of fluid blood indicating that blood clotting had occurred. Note obscuration of entire lower left lobe. *C*, Roentgen ray showing postero-anterior view of chest on November 17, 1st day postoperative (aspiration November 16, 31 days after injury). Note how decortication process performed at operation has allowed reëxpansion of lower left lobe.

S. aureus. It is not unlikely that the clot process is in the usual case materially hastened by both factors. Clotting may occur immediately succeeding the hemorrhage or may be delayed until a considerable period has elapsed. It is presumed that organization of the clot begins as soon as the clot has formed and proceeds until the process is more or less complete.

5. **Signs and Symptoms.** From the foregoing it will be recognized that the signs and symptoms of organizing hemothorax can be generally classified into 2 groups: (1) constitutional, resulting from body response from reaction within the pleura; manifestations vary considerably depending upon whether or not there is an infectious process present in the affected pleura coincident with the clot.

The localizing signs and symptoms are of considerably more significance. These arise in the main from pressure by the clot upon the lung and adjacent pleural structures, and vary in severity with the amount of involvement. Usually the chief complaint is that of dyspnea upon moderate effort, pain or a sense of oppression in the affected side, and persistent cough. Examination reveals limitation of respiratory motion over the affected side

with widening of the intercostal spaces when the contained clot is large and in the process of early organization. Tactile fremitus is decreased and the apex beat is shifted away from the affected side. Loss of normal pulmonary resonance and diminished vocal fremitus are present.

6. Diagnosis. The clinical signs are essentially those of fluid in the chest and except for the information available by Roentgen ray and aspiration, are indistinguishable from it. Usually chest films taken in the postero-anterior and lateral views show a diffuse haziness over the whole or part of the affected side of the chest with collapse of the lung of varying degrees. Air is commonly present but not invariably. The area of pulmonary collapse is frequently noted in the most dependent portion of the pleural cavity and affects the lower lobe. Early in the course of the lesion there may be, if sufficient fluid and air are present, widening of the intercostal spaces on the side of the lesion with a perceptible shift of the mediastinum to the side opposite. The fluid level, depending upon whether or not air is present, may or may not be sharply defined. As the process of organization proceeds, the fluid level which was formerly quite sharply delineated tends to become diffuse and hazy, the interspace of the thoracic cage narrowed, and the mediastinum shifted back toward the affected side. The diaphragm on the side of the lesion is in the later stages elevated above its normal position. Fluoroscopy will confirm a restriction of respiratory movement in the chest wall and particularly in the diaphragm. This restriction is due presumably to the pressure and tension produced by the progressive formation of adhesions as the clot begins to organize between the various pleural structures, lung, thoracic wall and diaphragm. Frequently loculi formed by organizing strands of fibrin and containing fluid are noted, and when present are indicative that clotting has occurred. It is of particular importance in cases suspected of developing organizing hemothorax to have lateral as well as antero-posterior Roentgen ray studies of the chest since exact localization and determination of the extent of the lesion are impossible without them. Furthermore, it should be emphasized that repeated Roentgen ray studies are essential to follow the progress of the lesion as well as any complicating features. These studies should be performed serially and rarely more than a week apart, both before and after surgical information.

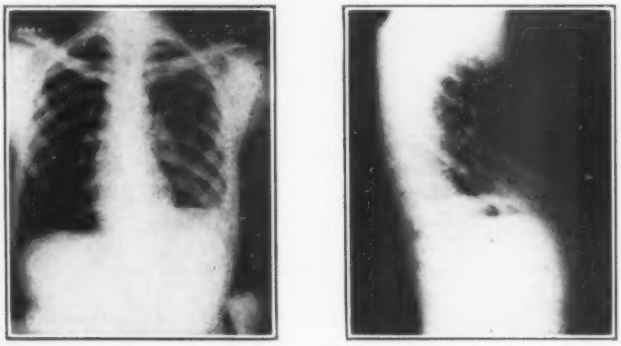

FIG. 4.—Roentgen ray showing postero-anterior and lateral views of chest at the time of discharge from the hospital on November 27 (11 days postoperative, 42 days after wounding). Note lung lobe has remained expanded and has progressively cleared.

Possibly the most important single diagnostic feature in establishing diagnosis of the lesion is findings on aspiration. In general it can be stated that when repeated aspiration of a pleural cavity showing clini-

cal signs and Roentgen ray evidence of a sizable collection of fluid shows negative results, a presumptive diagnosis of organizing hemothorax should be made. In fact, when no fluid is recovered from a site in the pleura where fluid is diagnosed from the extant clinical and Roentgen ray findings, it must be presumed that the fluid blood has become clotted and the condition of organizing hemothorax obtains. Nor is it necessary that no fluid whatever be obtained on repeated aspiration to establish the diagnosis. Very frequently small quantities of fluid blood may be recovered, but the important consideration is that the amount of fluid blood recoverable is minimal in comparison to the amount which should be recoverable by correctly performed aspiration in the absence of intrapleural clotting. It is seldom that the clotting process when it occurs in the pleura is entirely complete. Usually there exist small loculi containing laked fluid blood which may be aspirated if the containing loculi are directly penetrated by the needle. The fluid in most instances in laked blood may be seropurulent or frankly purulent in character when infection has occurred. It is obviously important to examine all aspirates bacteriologically for the presence and character of infective organisms. It is not uncommon, when small amounts of blood have been recovered during aspiration, to find small macroscopic clots present in the fluid. This is good presumptive evidence that a more extensive intrapleural clot exists. The appearance of strands of fibrin in the aspirate is also considered of similar significance.

In summary of the foregoing, it may be stated that the diagnosis of organizing hemothorax rests upon demonstration of the following features:

1. The existence of a penetrating intrapleural wound usually extensive in which a concomitant extravasation of blood would be expected.

2. Clinical signs and Roentgen ray evidence indicating the presence of fluid in the pleural cavity.

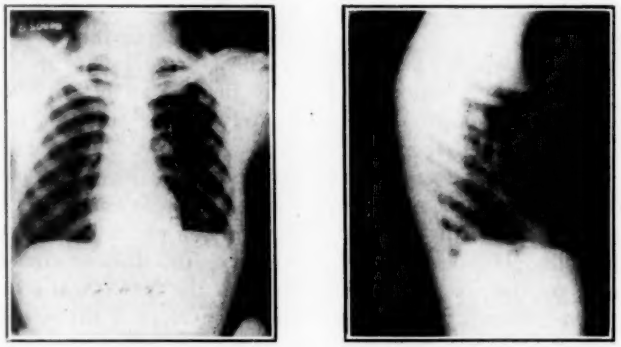

FIG. 5.—Case G. A. Roentgen ray showing postero-anterior and lateral views of chest on December 11 (2 weeks after discharge from hospital, 25 days postoperative, 59 days after wounding). Note that lung is practically normal in appearance.

3. Failure to recover fluid blood upon repeated aspiration or recovery of only small amounts containing macroscopic clot.

4. Signs and symptoms of lowered vital capacity due to lung compression.

5. The presence of pyogenic organisms in the pleural aspirate.

6. Specific Roentgen ray changes progressively revealed in serial films showing persistent shift of the mediastinal structures, elevation of the diaphragm, and haziness of a previously well-defined fluid level.

7. Considerations on Treatment. The treatment of organizing hemothorax may be classified into 2 general categories,

conservative or radical—the choice depending almost entirely upon the amount of anatomic and functional involvement. With minimal lesions in which only a small amount of lung parenchyma is concerned and the vital capacity of the individual only slightly reduced, a conservative regimen is advisable. Where the amount of involvement is small, considerable improvement takes place, leaving as a rule only a moderate amount of pleural thickening and little ultimate impairment of the function. The most frequent complication in such instances is the occurrence of empyema which should be watched for, and in the event of its incidence, be drained in the usual manner. Results under conservative treatment in properly selected cases are almost uniformly good.

It is in instances in which the lesion has involved a considerable amount of lung parenchyma with proportionate reduction in vital capacity and respiratory function, that radical surgical treatment must be considered, for, if the condition is allowed to continue without intervention, permanent pulmonary disability results proportional to the extent of the lesion. It has been arbitrarily stated by those whose experience in the treatment of organizing hemothorax has been considerable that, when the organizing clot has rendered functionless an estimated 20% or more of the functional lung tissue, radical surgical treatment should be instituted. Most of those observing the results in the treatment of a large number of cases are in general agreement with this concept. Once the decision to employ radical surgical treatment has been made, the time at which operation is to be carried out is a consideration of the greatest importance as will be apparent from the subsequent brief discussion concerning the technical procedure itself.

It is not within the scope of this writing fully to outline detailed surgical technique. There are, however, certain aspects of the operative procedure that bear such an intimate relation to the ultimate results that they cannot be conscientiously omitted. Pre-operative preparation is particularly important. Not infrequently the patient has coincident injuries which must receive treatment. It is unwise to attempt the requisite surgery until such time as the patient's general status is near the optimum point. Particular attention should be devoted to the elimination of all extant nutritional problems and infectious processes. The hemoglobin level should be approximately normal, and, if deficient, replenished by adequate transfusion. Immediately prior to operation, a quantity of whole blood (2000 cc.) should be available for transfusion during operation and the postoperative period.

8. Technique of Operation. The operative procedure itself consists in opening the pleural cavity, under endotracheal anesthesia, through a thoracotomy incision so placed that it will adequately expose the area involved by the organizing clot. The placement of this incision is previously determined by careful study of antero-posterior and lateral films of the chest. Occasionally it is possible to gain sufficient exposure by incision through the intercostal space but usually the removal of a rib is essential. Upon opening the pleura, particular care must be taken to avoid injury to any underlying lung which may be adherent to the chest wall. When the pleura is penetrated, the pocket, whose walls are formed of organizing clot and whose content is composed in most instances of serosanguineous or sanguinopurulent fluid and bits of free-floating fibrin, is exposed. The content of the pocket is evacuated by suction and sponging, and its limits and the character of its wall examined. The wall is then incised at some point over the underlying lung and by blunt and sharp dissection the visceral pleura of the lung is identified. If this maneuver is properly carried out a plane of cleavage is established between the visceral pleura and the overlying fibrous wall of the organizing clot so that the letter, by careful dissection, can be entirely stripped from the lung which it

compresses. The procedure is progressively continued until the entire area of lung compressed and encompassed by the clot is freed and the restricting wall of fibrous tissue removed. The lung is inflated under positive pressure by the anesthetist to test its expansibility and air continence and the pleural cavity thoroughly lavaged with normal saline to remove residual blood. Drains are inserted in the dependent portion of the pleural cavity and these are immediately connected to water seals. The chest is closed in the usual manner, if the procedure has been properly performed, the lung, which had been previously compressed, reëxpands completely and remains so, and the individual thereby regains practically the entire amount of functional lung tissue.

9. Special Features Concerning the Operative Treatment. Several features concerning the actual surgical procedure are noteworthy. It has already been stated that the time after injury at which operation is carried out is of considerable importance. This is based on the fact that once the clot is formed, it becomes progressively more organized by the ingrowth of fibrous tissue from adjacent pleural structures. If operation is carried out too soon, that is, before there has been sufficient ingrowth of fibrous tissue, the procedure of peeling or decorticating the lung is made technically extremely difficult, due to the fact that the clot is too friable and separates away from the visceral pleura in very small strips. If, on the other hand, the organizing process has been allowed to progress for too long a period, the overlying clot becomes too firmly adherent to the visceral pleura of the lung and separation causes injury to the visceral pleura and underlying lung, and is attended by far more hemorrhage. In either case the establishment of a plane of cleavage between the clot and visceral pleura is extremely difficult and the decortication procedure rendered much more complicated. The time at which decortication should be performed has been a matter of much speculation. It has been more or less established empirically that the optimum time for operation in the usual case is somewhere between the 3rd and 5th week after clotting has been shown to have occurred. Under ordinary circumstances the decortication performed within this period will result in the establishment of a satisfactory line of cleavage between the clot and the visceral pleura so that the technical process is not too difficult. Great care must be exercised to avoid injury to the vital structures contained in the mediastinum, particularly the great vessels. If the lung parenchyma is inadvertently penetrated as it frequently is during the procedure, the area of parenchymal penetration should be closed at once with the fine silk suture.

Before closing the chest, the surgeon should see that the lung is entirely expanded and fills all of the pleural space. Lavage of residual hemorrhage incident to the peeling process is important and has been mentioned elsewhere. The establishment of adequate drainage is important. This can usually be effectively accomplished by the proper placement of a fenestrated rubber tube of medium caliber in the posterior pleural gutter, and a secondary drainage tube consisting of a catheter placed through the anterior chest wall into the pleura. The fenestrated tube serves to remove accumulations of fluid while the catheter provides a conduit for any remaining air. In each case the tubes should be immediately connected with water seals to allow the lung to become functional as soon as possible. It is the general practice to remove the tubes when there is no longer any fluctuation noted in the water seals.

10. Postoperative Complications. The patient must be carefully watched for postoperative complications. Empyema is a frequent complicating factor which if it appears is treated by drainage in the usual manner. Bronchopleural fistula may occur in which event subsequent operation may be necessary for closure. By far the most frequent untoward postoperative sequel in atelectasis

of the affected side and every effort should be made to forestall its appearance. This can, in most instances, be accomplished by seeing that the drainage tubes are kept clear and by preventing accumulation of secretion in the bronchial tree. In the event that postoperative atelectasis does supervene, bronchoscopy and aspiration usually are necessary. In the majority of instances, however, the procedure of decortication in carefully selected cases of organizing hemothorax should yield uniformly good results.

11. Case Presentation. The following case of organizing hemothorax is presented as showing the typical features with regard to diagnosis, radical surgical treatment and ultimate result. The accompanying illustrations are taken from this case.

G. A., a 19 year old negro male, was admitted Oct. 22, 1945, to the University Surgical Service of the Detroit Receiving Hospital with a stab wound of the left chest. Examination on admission showed that the wounding instrument, a knife, had penetrated the chest wall in the left scapular line at the level of the fourth interspace. Chest findings confirmed by Roentgen ray revealed the presence of a left hemothorax. Patient was put on observation and conservative treatment. Later, on the day of admission, he complained of dyspnea and the left chest was aspirated of 650 cc. of blood with improvement of symptoms. On October 29, his chest was reaspirated and 850 cc. of laked blood recovered. On November 3, his temperature, pulse and respirations were elevated and reaspiration of the chest recovered 350 cc. of bloody fluid. Cultures returned upon previously aspirated fluid showed the presence of a non-hemolytic streptococcus and *S. albus*. The patient continued to run a fever in spite of the administration of penicillin in large doses. Roentgen ray studies showed the presence of fluid but none was recovered on 3 successive aspirations. A tentative diagnosis of organizing hemothorax involving the left lower lobe was made and the patient prepared for surgery. The operation was performed on November 16, 25 days after being wounded.

The pleural cavity was approached through a standard thoracotomy incision by removal of the sixth rib. The entire left lower lobe was found to be tightly compressed against the mediastinum by an extensive organizing clot whose walls were composed of ingrowing connective tissue and whose control portion contained laked blood and many light adhesions. The compressed lower lobe of the left lung was completely decorticated, leaving a practically normal visceral pleura beneath. Subsequently the lung was expanded under positive pressure and filled the entire pleural cavity. Drains were inserted and the chest closed. The entire procedure lasted 2 hours and 45 minutes. The patient received a 500 cc. transfusion of whole blood during operation and left the operating room in good condition.

The postoperative course was uneventful. The lung remained well expanded as shown by interval Roentgen rays. Drains were removed on the 4th postoperative day. The patient shortly thereafter became ambulatory and practically asymptomatic. He was discharged to the out-patient department on December 28. When last seen in the out-patient department, on Feb. 22, 1946, he was asymptomatic and had resumed his full normal occupational activity.

REFERENCE

TUTTLE, W. M., LANGSTON, H. T., and CROWLEY, R. T.: The Treatment of Intrathoracic Wounds, Surg., Gynec. and Obst., **81**, 158, 1945.

BOOK REVIEWS AND NOTICES

THE AUTONOMIC NERVOUS SYSTEM. By ALBERT KUNTZ, PH.D., M.D. Third ed. Pp. 687; 91 illus. Philadelphia: Lea & Febiger, 1945. Price, $8.50.

OF the relatively few books on the autonomic nervous system, this has long been regarded as the best. Research along this line has been progressing so rapidly that already many views are changing. Nevertheless, Dr. Kuntz has nicely reviewed the literature up to 1942 and has integrated the mass into a readable story.

The general outline includes, first, general anatomic and physiologic considerations, second, a detailed review of the systems supplied by the autonomics and, third, the rôle of these nerves in the pathogenesis of various diseases. Lastly he reviews the surgical approaches and attempts an evaluation of their efficiency.

The 100-page bibliography indicates the labor spent in preparing this volume. Because of this list it is obvious that the book is a good reference text. J. C.

THE EXTREMITIES. By DANIEL P. QUIRING, PH.D., Head of the Anatomy Division, Cleveland Clinic Foundation and Associate Professor of Biology, Western Reserve Univ.; BEATRICE BOYLE, Artist, Cleveland Clinic Foundation; ERNA L. BROUSH, M. A., Anatomy Division, Cleveland Clinic Foundation; and BERNARDINE LUFKIN, A.B., former Secretary, Research Division, Cleveland Clinic Foundation. Pp. 117; 107 illus. Philadelphia: Lea & Febiger, 1945. Price, $2.75.

IN order to simplify the relations of the muscles, nerves and arteries of the upper and lower extremities, the authors have depicted 101 muscles, each in a separate diagram. In each diagram, besides the origin and insertion of the muscle the chief arterial and nervous supplies are shown. On 92 of the muscles the motor points of the muscles as found in normal subjects are presented. The plates of the separate muscles will be welcomed by medical students and will serve as a quick source of reference to all concerned with function and dysfunction of the limbs. The position of the motor points should be especially useful to physical therapists. W. A.

THE CHEMISTRY OF ANESTHESIA. By JOHN ADRIANI, M.D. Pp. 502; 45 illus. Springfield: Thomas, 1946. Price, $7.00.

ANESTHESIOLOGY is coming of age as a medical specialty. This seasoning process has been accomplished in part by books concerned with various fundamental aspects of the field. There are volumes devoted to the physiology of anesthesia, and to the pharmacology of anesthesia. Here now is the chemical approach to problems of anesthesia.

Adriani has done a splendid job on the whole. There are lucid presentations of such important topics as carbon dioxide absorption, the application of physical laws of gases to clinical anesthesia, explosion hazards, basic chemistry of such substances as ethers, aldehydes, estrus, etc. The book is divided into 3 large sections: inorganic chemistry, organic chemistry and biochemistry. In the latter 2 sections there is a good bit of pharmacology; but, as the author points out, this is inevitable.

There is nothing new in the volume. It is merely a compilation of information designed for the information of clinical anesthetists. As such it has an appeal limited primarily to this group. For them, however, it can be unhesitatingly recommended. R. D.

PATHOLOGY IN SURGERY. By NATHAN CHANDLER FOOT, A.B., M.D., Professor of Surgical Pathology, Cornell Univ. Medical Coll.; Surgical Pathologist, New York Hosp. Pp. 512; 368 illus. Philadelphia: Lippincott, 1945. Price, $10.00.

"THIS book is intended as a guide to the surgical pathology of those disorders in which operations are carried out and organs or other specimens removed . . ." It

can serve as a text for a student course in surgical pathology or as a handbook for those training in pathology. There are few good textbooks in this special field and a new one is a welcome addition.

The book begins with a discussion of the methods used in surgical pathology, followed by chapters on the general pathology of inflammation, wound healing, and tumors. The largest part of the book is devoted to the special pathology of various tissues and organs: fibrous tissue, cartilage and bone, bone marrow, lymph nodes and spleen, alimentary tract, breast, nervous system, and skin—to list a few examples. Important lesions are discussed in considerable detail, others are merely mentioned. As the author states, the book is not intended to "be too comprehensive in the theoretical consideration of all the aspects of the lesions that are described." Adequate bibliographies are given for those desiring to pursue the details of any special subject.

Practically all of the illustrations are original. They are of very uneven quality. A few are excellent (notably those from the Army Medical Museum), most are fair to poor. The color reproductions are unbelievably bad. Most are unrecognizable even with the legends and the book would be greatly improved by the omission of many. One serious error is the inclusion of some photographs of breast tissue twice, once to illustrate intraductile papillomas, once to illustrate malignant change.

The book is published in the new double-column style for easy reading and is bound as a companion to Karsner's "Human Pathology." It will surely prove useful to students and pathologists alike.

W. S.

COLLOID CHEMISTRY, THEORETICAL AND APPLIED. Collected and edited by JEROME ALEXANDER. Vol. VI. General Principles and Specific Industries, Synthetic Polymens and Plastics. Pp. 1215; fully illus. New York: Reinhold, 1946. Price, $20.00.

THIS 6th volume of Dr. Alexander's encyclopedia of colloid chemistry contains 71 contributed papers by experts in their chosen fields. The subjects are approximately evenly distributed between applications of colloid chemistry in industrial and technologic processes and the enlarging fields of resins and plastics.

Although even plastics have some biologic interest (with reference to proteins), among the chapters of more direct interest to medicine and biology are excellent discussions of the "Mass Spectromotor and Its Applications" (containing many interesting applications besides those of isotopes in the study of intermediary metabolism) and "Some Practical Aspects of Electron Microscopy." The latter article by Drs. V. K. Zworykin and J. Hillier is exhaustive and well illustrated. There is also a comprehensive review by Dr. F. C. Combes on "The Skin and Its Technical Hazards," which should be of particular interest to dermatologists. Other subjects which may prove useful to biologists are "Adsorption from Solution by Activated Carbon" and "Removal and Recovery of Proteins by Water-soluble Lignin."

As a timely inclusion there is a final chapter on nuclear energy and atomic fission. This is a very readable account, contributed by the editor.

Alexander's "Colloid Chemistry" from its inception in 1926, when Volume I was published, has been recognized as a standard work. The individual articles are valuable and authoritative. However, the time has been reached when it would seem desirable to publish an index volume, since it has become practically impossible to "readily place one's hand" upon a particular subject in this large reference work. While no doubt little can be done about it, *opera* such as these appear destined only for the shelves of relatively large libraries—$20 a volume for a periodically published work is not an easy cost for the average interested reader.

D. D.

BIOENERGETICS AND GROWTH. With Special Reference to the Efficiency Complex in Domestic Animals. By SAMUEL BRODY, PH.D., Chairman, Committee on Growth and Energy Metabolism College of Agriculture, Univ. of Missouri, Columbia, Mo. Pp. 1023. New York: Reinhold, 1945. Price, $8.50.

THE present work by Dr. Samuel Brody, an active member of the research staff of an agricultural and animal husbandry experiment station, is concerned with the inter-

related subjects of growth, efficient utilization of energy and the processes of aging in farm animals. The fields covered by the book are dominant trends in recent biologic investigation, making the treatise very timely.

The farm animals, fowl, hog, cow and horse, have been neglected owing to their size as laboratory subjects, but they should be of particular interest as producers of foodstuffs—egg, milk, meat—as producers of muscular work (the horse), and, finally, as sources (the bovines) of hormones for replacement therapy in man. Dr. Brody's volume is an integrated compilation of data secured over many years in a coöperative effort by the staff of the Missouri Agricultural Experiment Station (Univ. of Missouri), made possible by endowment by the Herman Frasch Foundation for Research in Agricultural Chemistry. The writing of the volume was aided by a fellowship grant of the John Simon Guggenheim Memorial Foundation.

The underlying theme is the farm animals as energy consuming and producing machines. Such subjects as animal calorimetry, surface area (of cattle), growth, production of milk, etc., and production efficiency correlated with aging are discussed in detail. Numerous tables and figures make for a well documented, encyclopedic text, not too easily read.

The whole work bears the stamp of authority, though somewhat biased by personal viewpoint. Dr. Brody's scholarly volume appears destined to serve as a source of information in this important field of "bioenergetics"—growth and production efficiency in farm animals, and is worthy of high commendation. D. D.

Roentgen Diagnosis of Diseases of the Gastrointestinal Tract. By John T. Farrell, M.D., Clinical Professor of Radiology, Graduate School of Medicine, Univ. of Penna.; Radiologist, Lankenau Hosp. Pp. 271; 190 illus. Springfield, Ill.: Thomas, 1946. Price, $5.50.

This book is a manual which will serve as a guide to some of the procedures carried out in the examinations of the various portions of the gastro-intestinal tract. The standard nomenclature of diseases is utilized and the author has arranged a brief discussion of the various lesions of the tract. As the author states in the Preface, foreign bodies in the gastro-intestinal tract have been emphasized because of his unique experience in that field. It is not a comprehensive treatise. As a small monograph on certain phases of diagnosis or treatment it follows the general trend of many of the more recent medical publications. With the text the author has collected a group of excellent illustrations.

The publisher is to be congratulated no the excellence of the physical qualities of the book. Its composition, the excellent paper, the print and the illustrations maintain the high standard that Mr. Thomas has set for his books. E. P.

Tratado de Cardioangiologia. By Pedro A. P. Tapella. Pp. 946. Lopez & Etchegoyen, SRL, 1946.

This well-documented volume presents in considerable detail the subject of cardiovascular diseases in 3 large divisions. More than a third of the book is given to generalities of cardiovascular anatomy, physiology and semeiology; a full half of the space is occupied by Part 2, The Cardiopathies; the 94 pages of Part 3 are given to Treatment. The subdivisions are much as one would find in a similar work in English. The 2066 references in the bibliography are to American articles in such a large proportion that those nationalistically minded will be much pleased. They should not assume, however, that this is necessarily a true index of the importance of American contributions to the subject and not merely indicating, perhaps, the current urge for writing and the opportunity for publication in this country. E. K.

Western Reserve University Centennial History of the School of Medicine. By Frederick Clayton Waite. Pp. 588; 16 illus. Cleveland, O.: Western Reserve Univ. Press, 1946. Price, $6.00.

Dr. Waite's book is the first history to be written of this important School of Medicine. It will necessarily be of especial interest to its friends and graduates and also is a valuable local record. He has gone

further, however, than portraying the institution with which he has been connected for more than half of its existence. The first 4 chapters give a brief survey of early medical instruction in this institution; other chapters consider rival institutions, and 9 appendices that of diverse matters. In fact, as the events portrayed are also considered in their causal and relative relations, the book may be regarded as covering much of the history of medical education in Ohio.

E. K.

CLINICAL ELECTROCARDIOGRAPHY. By DAVID SCHERF, M.D., F.A.C.P., Associate Professor of Medicine; and LINN J. BOYD, M.D., F.A.C.P., Professor of Medicine, New York Medical Coll., Flower and Fifth Ave. Hosps. 2nd ed. Pp. 267; 243 illus. Philadelphia: Lippincott, 1946. Price, $8.00.

THE authors state that in preparing this edition they have been primarily interested in a close correlation between electrocardiographic findings and their clinical evaluation and that for this reason emphasis has been directed to the practical applications of electrocardiography, to the clinical aspects of disturbances of rhythm and to therapy.

The discussion of abnormalities of cardiac mechanism is excellent and may be read with profit by the beginner and with interest by those who have had wide experience in this field. The only adverse comment this Reviewer can offer concerning this part of the book is that a few of the tracings made with the machines of the past generation do not reproduce well enough to be informative.

With regard to those sections which deal with the most important aspects of clinical electrocardiography, such as the changes produced by intraventricular conduction defects and various forms of myocardial damage incident to coronary disease, the book leaves much to be desired. The authors have apparently adhered to the one precordial lead school of thought and very few of the illustrations include more than one lead of this type. The inadequacy of the 4 lead electrocardiogram for the display of bundle-branch block and lesser grades of intraventricular conduction defects as well as localized areas of damage involving certain parts of the ventricular wall presenting toward the anterior surface of the chest has apparently not been recognized. Likewise the importance of the patterns of change that may be recorded when leads are made with the exploring electrode placed on an adequate number of precordial positions has not been stressed. Thus, in certain important respects, the book is not abreast of current thought and practice in electrocardiography.

C. W.

THE SULPHONAMIDES IN THEORY AND PRACTICE. By J. STEWART LAWRENCE, M.D. (EDIN.), M.R.C.P., Physician, Haymeads Emergency Hosp., Bishop's Stortford. Pp. 105; Preface and Bibliography. London: H. K. Lewis, 1946. Price, 9/.

A BRIEF summary of the history, mode of action, pharmacology, clinical use, toxicity, and present status of these drugs, including a bibliography of 323 titles and directions for estimating the sulfonamide content of blood or urine. It is written mainly for the guidance of clinicians and therefore presents for each drug such items as structure, absorption, excretion and distribution in the body, which are not usually available in as concise form as this. American observers may not be ready to accept the author's conclusion that sulfamethazine is the sulfonamide of choice for routine use but the descriptions of clinical use, toxic symptoms, and choice between these drugs and penicillin are worthy of highest commendation.

C. S.

NEW DRUGS. By ARTHUR D. HERRICK. With a Foreword by DR. AUSTIN E. SMITH. Pp. 295. New York: Revere, 1946. Price, $4.00.

THIS book is intended to describe and explain the requirements of the Federal Food, Drug and Cosmetic Act of 1938 with regard to the testing of new drugs before they are marketed to the public. The author, evidently a lawyer with considerable experience in this field, sketches the background and scope of the present law interestingly and informingly in 15 chapters (150 pages), and adds an Appendix (138 pages) containing the documents relative to the notorious Elixir Sulfanilamide Massengill, the text of the present Federal law, pertinent excerpts from present state and city laws, and the official rules of the Council on Pharmacy and Chemistry of the

American Medical Association, whose secretary contributed the Foreword. The book should be extremely valuable to those concerned with the making and testing of new drugs. The average physician may also read with profit this account of some of the past shortcomings of his own profession with respect to the use on unsuspecting patients of insufficiently tested new drugs.

C. S.

HOWELL'S TEXTBOOK OF PHYSIOLOGY. Prepared by JOHN F. FULTON. 15th ed. Pp. 1304. Philadelphia: Saunders, 1946. Price, $8.00.

A NEW edition of this classic is indeed welcome after the lapse of 6 years. The present edition, the first since Dr. Howell's death, has been prepared by Dr. John Fulton with the help of 10 collaborating editors. It shows, as one might expect, an extensive revision with many new and rewritten chapters. Though this has required 187 more pages, the book is not inconveniently larger or heavier. Six of the opening pages are given to a preliminary statement on Historical Background of American Physiology; one might wish that this was several times as long and not limited to "American," especially as it is contributed by the editor. The strength of the Yale faculty in physiologic activities may be inferred from the fact that 15 of the 24 contributors are members of that faculty and all but 3 of professional grade. Though the Preface gives some idea of the main changes, one must study the book itself to appreciate them. E. K.

ESSENTIALS OF ALLERGY. By LEO H. CRIEP, M.D., Assistant Professor of Medicine and Lecturer in Immunology, School of Medicine, Univ. of Pittsburgh, etc. With a Foreword by ROBERT A. COOKE, M.D., Chairman, Committee on Education, American Academy of Allergy. Pp. 381; 42 illus., 1 color plate. Philadelphia: Lippincott, 1945. Price, $5.00.

THIS handy little volume fully lives up to its title. Allergy is a rapidly growing field touching every phase of medical practice, but including much that is as yet controversial. A full statement of the subject is far too involved for any except those working in the field. On the other hand, current textbooks in medicine, surgery and the specialties are inadequate in their presentation of allergy. There has heretofore been a need for a concise and practical text for the use of students and physicians: a need which this volume fully meets. The author has shown excellent judgment in the type and amount of material included and a commendable clarity and order of presentation. There are chapters on the basic principles of anaphylaxis and allergy: the diagnosis and treatment of allergy; hay fever, asthma, nasal and cutaneous allergy; allergy to sera, drugs, bacteria, fungi, physical agents; miscellaneous manifestations of allergy; allergy in children; the diagnostic skin tests for various diseases. There are many illustrative case reports. As Dr. Cooke says in his Foreword, the book "should be a joy to the undergraduate students and the general practitioners for whom it is intended."

R. K.

ASCLEPIUS. A Collection and Interpretation of the Testimonies. Book I. Testimonies and Book II. Interpretations. By EMMA J. and LUDWIG EDELSTEIN. Pp. 479 (I) and Pp. 1304 (II); no illus. Publications of the Institute of History of Medicine, 2nd Series, and Documents, Vol. II. Baltimore: Johns Hopkins Press, 1946. Price, $7.50 for Book I, and $8.00 for Book II.

THIS painstaking, scholarly work of the 2 Edelsteins is one of the most monumental that has thus far emerged from the Johns Hopkins Institute. It aims at understanding the problem of Asclepius, both in his aspect of the divine healer and his later rôle, in the final stage of paganism, as "the foremost antagonist of Christ" in the early days of Christianity. The first and larger volume is a Collection of the Testimonies, *i. e.*, Greek and Latin references (fortunately for most of us, with English translations included). They are arranged in 7 categories: (1) Legend (his birth, accomplishments, life character and death, his tomb, and his star, Ophiucus); (2) Descendants; Deification; Medicine; Cult; Images; Sanctuaries. An 18 page *Index Locorum* offers ready reference to the sources. The second volume, the Interpretation of the Testimonies, "aims at an insight into the essence of the Asclepius figure and of the cult attached to him," and also provides a commentary on the "testimony" of the

ancients. The authors stick closely to their subject; they have aimed to avoid such tempting considerations as the nature of the ancient deities, or Asclepius' place among them, comparison of Greek and Roman views with those of other nations, theories of the origin of religion and similar related topics. Asclepius, himself, however, is considered in his human rôle up to classical times and as a god thereafter, also his cult, his images, his temples. Much that has seemed incongruous to the average reader of Greek medical history finds explanation in this entertaining volume and the wealth of scholarly observation and interpretation will make it of permanent value to other scholars. We congratulate heartily the Johns Hopkins Institute on the appearance of this noble product. E. K.

HIPPOCRATIC WISDOM. By WILLIAM F. PETERSEN, M.D. Pp. 263; 77 illus. Springfield, Ill.: Thomas, 1946. Price, $5.00.

THE laudable purpose of Dr. Petersen's book is to bridge the chasm between the technical dexterity that our recently acquired wars of objective information permit and the integration of the sum of medical knowledge that the patient requires. This, he thinks, can best be done by returning to Hippocrates. His well-known concern with the effect of environmental influences on man naturally led him to Avis Waters Places, which with some justification he regards as the high point of Hippocrates' objective study of disease. Whether he has read into the text more than the author had in mind, and whether it is correct in appraisal of disease that "the weather comes first," that is indeed another matter. Nor will all accept that Hippocrates was the first meteorologist, the first anthropologist, ecologist and biologic scientist.

By always presenting Hippocrates' own words in roman type, the bulk of the book is in italics. This method brings out various pomp which none but the close student of Hippocrates is apt to realize. For instance, though the Greeks are not regarded as having performed autopsies, Hippocrates is shown to assert that from the dead we know the living and to have recognized a relation between tubercles in the lungs and "curvature and contraction" of the spine. We recognized that the seat of disease had importance, that swimming creatures had to inhale the air in the water, that "male seed passes into the uterus and there commingles with the female seed," and so on through many more examples. Anoxia, reproduction, the medical and surgical clinics, and even theory receive illuminating treatment, which the Reviewer confesses is much more impressive than he had anticipated. This short book has a far greater value than that of entertainment for the historically minded reader. It can advantageously be studied by those medical neophytes who want a perspective for their detailed studies. E. K.

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